Affymetrix®

GeneChip® Operating Software

With AutoLoader

User's Guide

Version 1.1

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701439 Rev. 2

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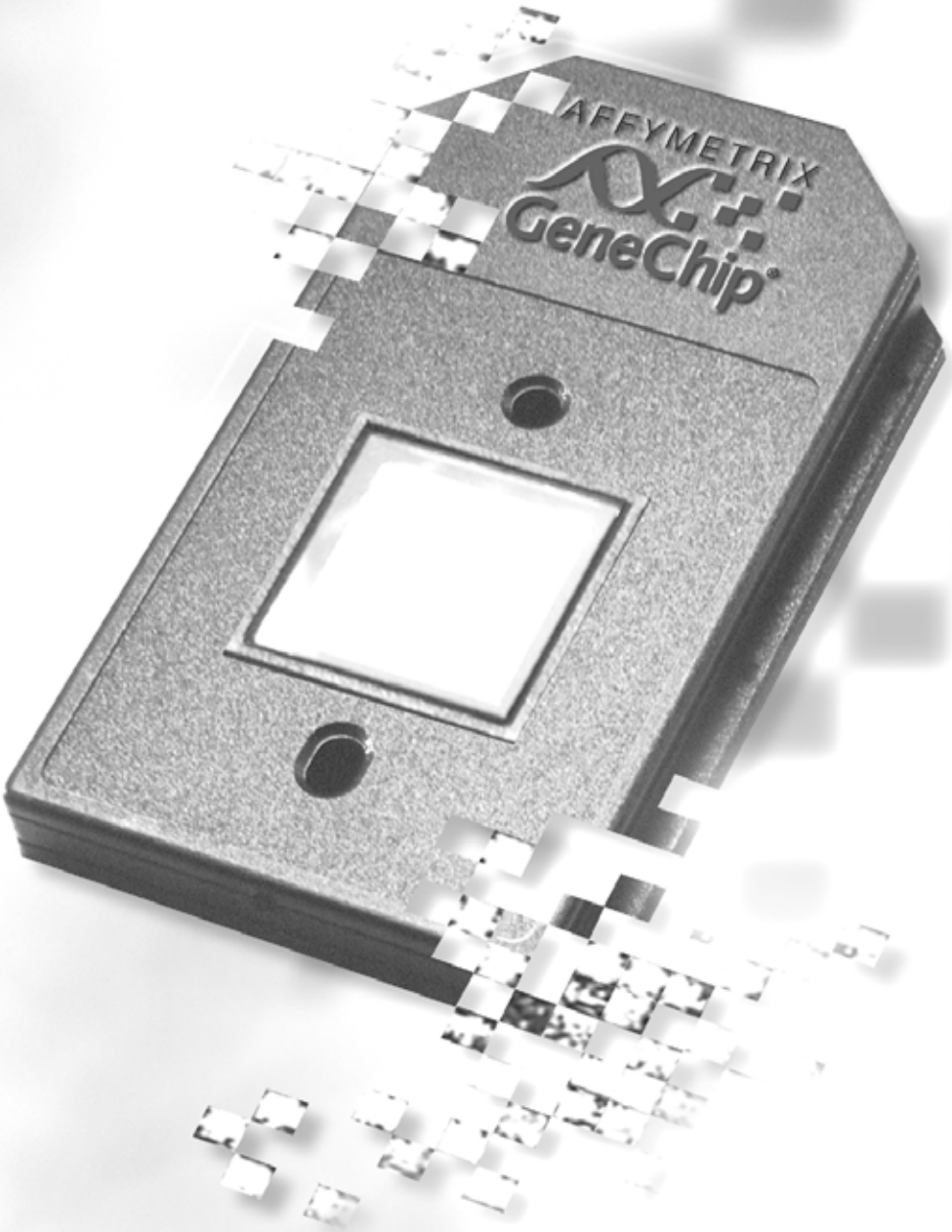

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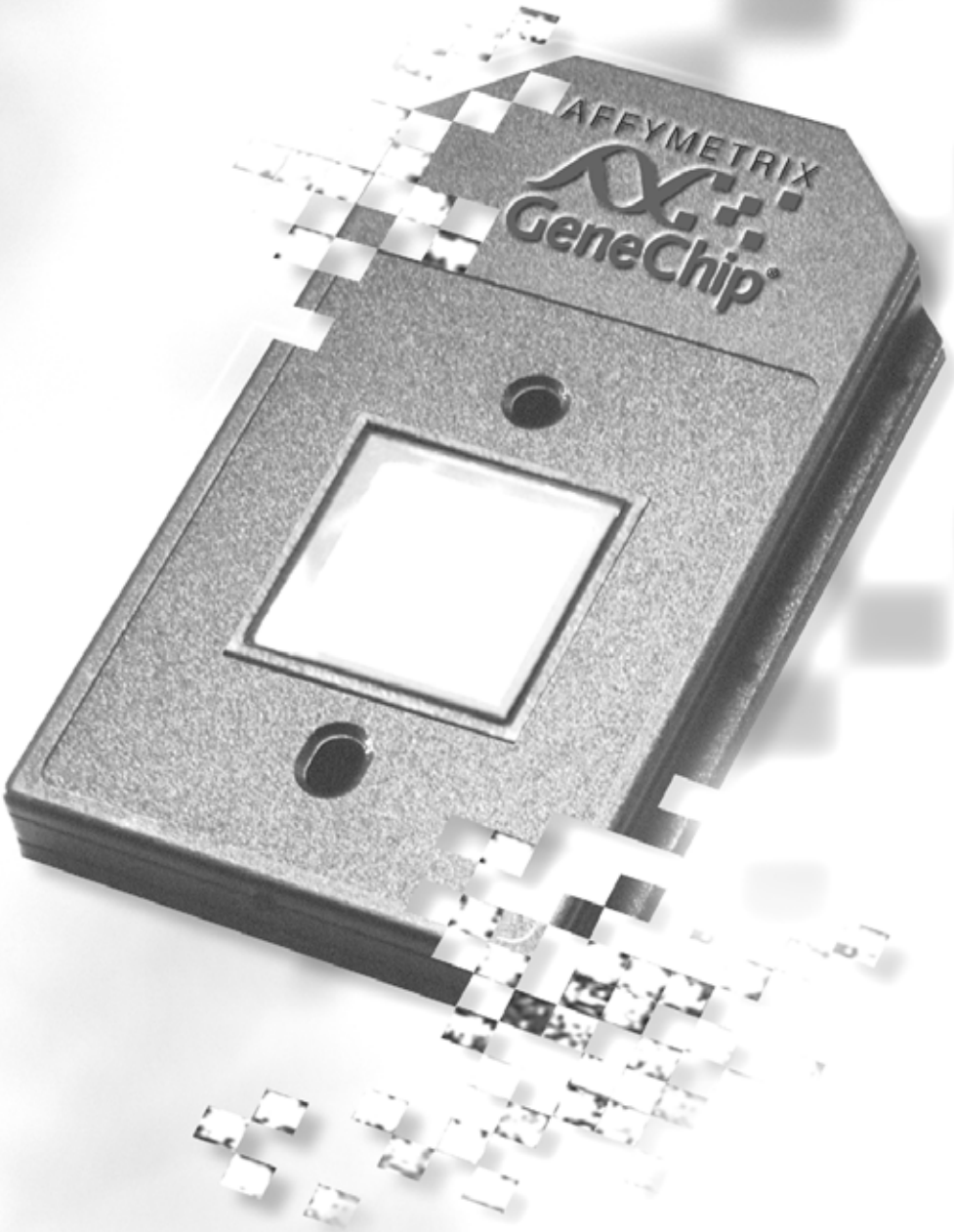
Chapter 1



Welcome to the GeneGhip® Operating System

Welcome to the Affymetrix® GeneChip® Operating Software 1.0 User's Guide. This manual explains how to use the following applications:

- GeneChip® Operating Software (GCOS) 1.0 (formerly Affymetrix® Microarray Suite)
- GCOS Manager (formerly Affymetrix® LIMS Manager)
- GCOS Administrator

Introducing GeneChip® Operating Software

GCOS 1.0 manages Affymetrix® GeneChip® array data. GCOS 1.0 manages data on the local work station using a Microsoft® Data Engine database on the workstation. GCOS also has the ability to connect with Affymetrix® GeneChip® Array Operating Software Server, a separate application for large scale, network accessible databases.

GCOS, GCOS Manager, and GCOS Administrator are a trio of applications that:

- automate control of the GeneChip® instruments
- capture and analyze the array image
- provide workflow tracking of experiment data (image, cell intensities, probe analysis data)
- manage experiment data
- automate basic expression analysis and publishing
- provide end user administration tools for workstation databases

Conventions Used in This Guide

This manual provides a detailed outline for all tasks associated with Affymetrix® GCOS, GCOS Manager, and GCOS Administrator. Various conventions are used throughout the manual to help illustrate the procedures described. Explanations of these conventions are provided below.

Steps

Instructions for procedures are written in a step format. Immediately following the step number is the action to be performed. On the line below the step there may be the following symbol: ⇒ This symbol defines the system response or consequence that happens as a result of user action; what you see and what has happened.

Following the response additional information pertaining to the step may be found and is presented in paragraph format. For example:

9. Click **Yes** to continue.

   ⇒ The Delete task proceeds.
   In the lower right pane the status is displayed.

   To view more information pertaining to the delete task, right-click **Delete** and select **View Task Log** from the shortcut menu that appears.

Font Styles

Bold fonts indicate names of commands, buttons, options or titles within a dialog box. When asked to enter specific information, such input appears in italics within the procedure being outlined.
For example:

1. Click the **Find** button or select **Edit** →**Find** from the menu bar.

   ⇒ The Find dialog box appears.

2. Enter *AFFX-BioB-5_at* in the **Find what** box, then click **Find Next** to view the first search result.

3. Continue to click **Find Next** to view each successive search result.

Screen Captures

The steps outlining procedures are frequently supplemented with screen captures to further illustrate the instructions given. The screen captures depicted in this manual may not exactly match the windows displayed on your screen.

Additional Comments

TIP	*Information presented in Tips provide helpful advice or shortcuts for completing a task.*
Note	*The Note format presents important information pertaining to the text or procedure being outlined.*
! CAUTION	*Caution notes advise you that the consequence(s) of an action may be irreversible and/or result in lost data.*
WARNING	*Warnings alert you to situations where physical harm to person or damage to hardware is possible.*

Resources

Online Documentation

The CD with GCOS includes an electronic version of this user's guide. The online documentation is in Adobe Acrobat® format (a *.pdf file) and is readable with the Adobe® Acrobat Reader® software, available at no charge from Adobe at *http://www.adobe.com*.

The online help contains a version of this user's guide as a Microsoft® HTML help file.

Technical Support

Affymetrix provides technical support to all licensed users via phone or E-mail. To contact Affymetrix® Technical Support:

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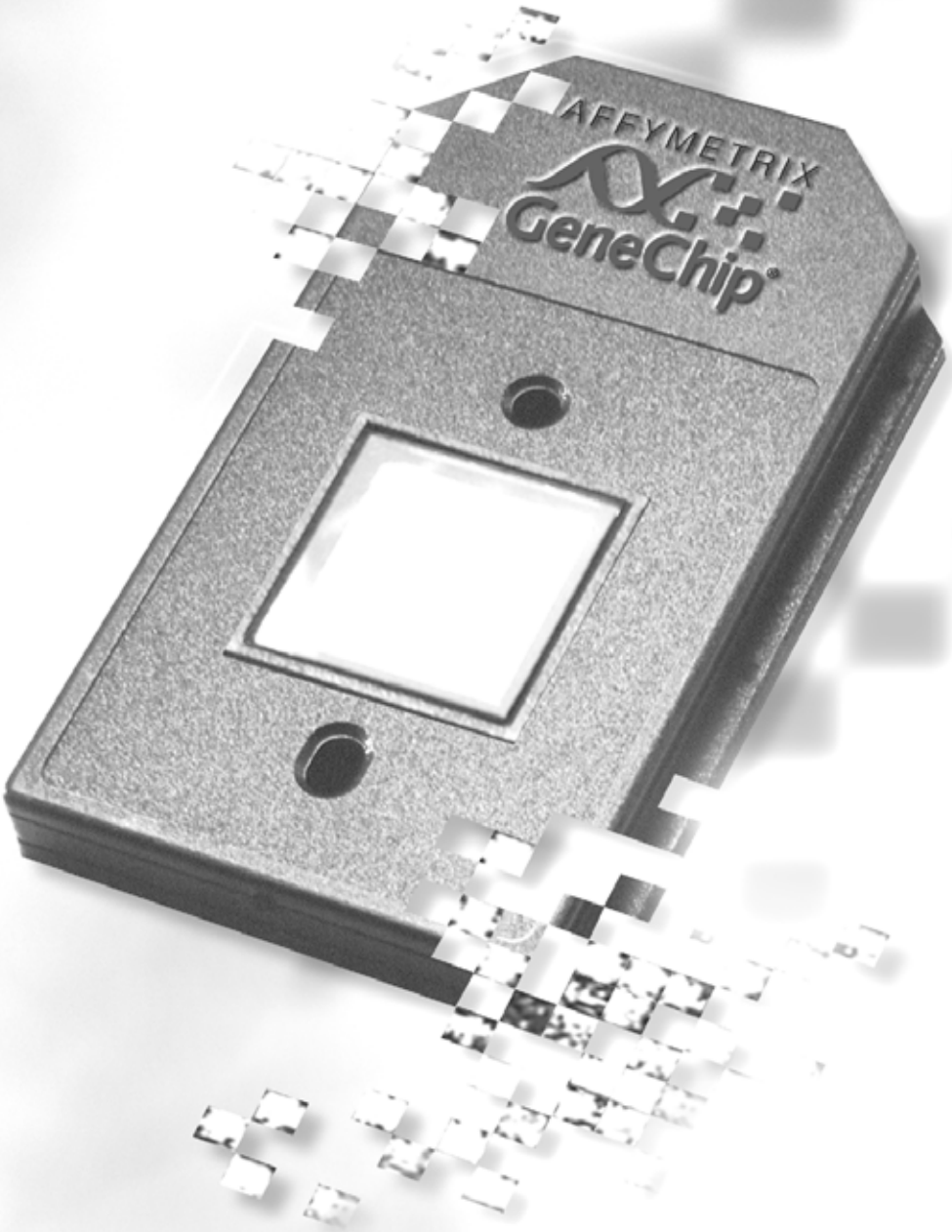

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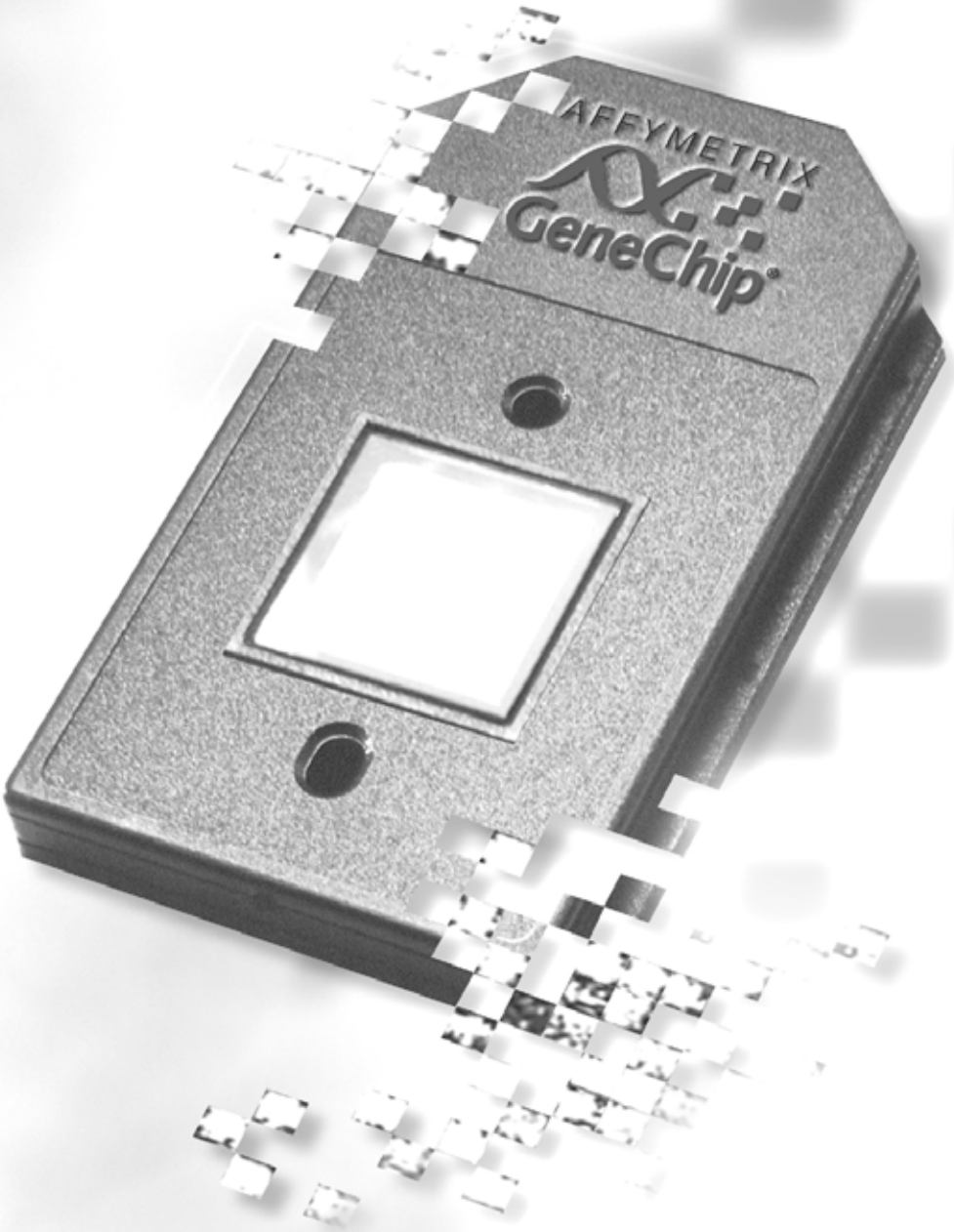
Chapter 2



Installing GCOS

This chapter provides detailed instructions for installing the GeneChip® Operating Software (GCOS) for new users and for users upgrading from Affymetrix® Microarray suite 5.x.

For information on installing the library files, see Chapter 3, *Affymetrix® GeneChip® Library Install*, on page 25. *For information on installing the instruments, see* Appendix C, *GCOS Instrument Installation*, on page 467.

Steps to Install GCOS for New Users

Note

You must be logged in as an administrator to install GCOS.

The screen captures depicted in this manual may not exactly match the windows displayed on your screen.

At least 500 MB of available disk space is recommended for the installation.

1. Insert the GCOS CD-ROM.
2. If the autorun feature does not start the program:
   a. Click **Start** →**Run**.
   b. Type <*cd drive letter*>:*setup.exe*.
   c. Click **OK**.

   ⇒ The Affymetrix Software Setup window appears **(Figure 2.1)**.

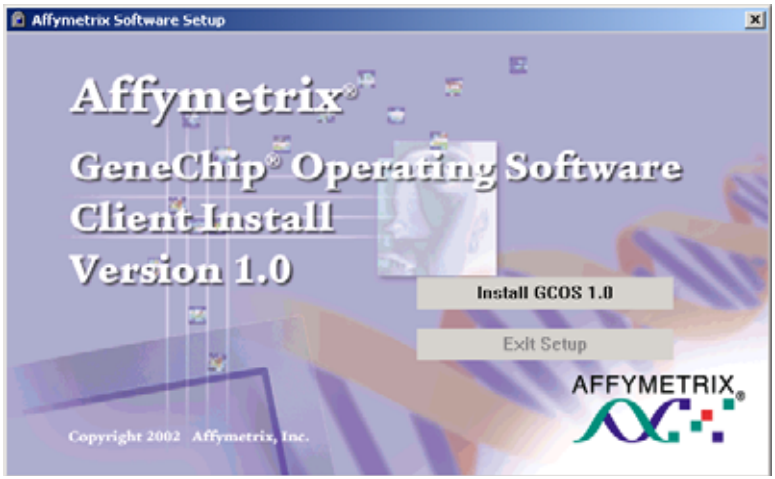


Figure 2.1
Install window

3. Click **Install GCOS**.
4. In the Welcome window **(Figure 2.2)**, click **Next**.
   ⇒ The License Agreement windows appear **(Figure 2.3)**.

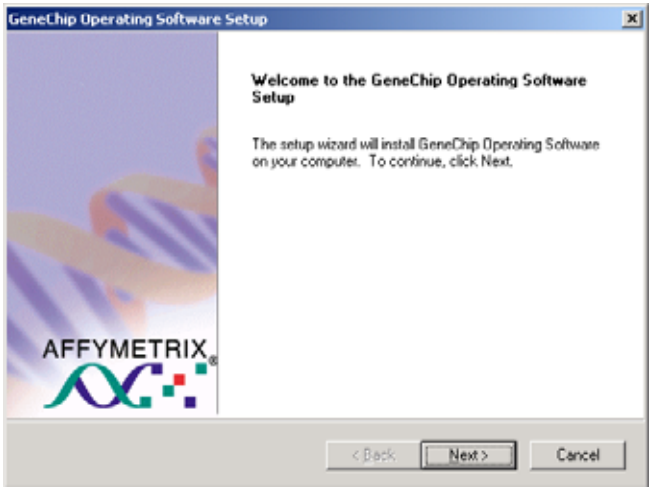


Figure 2.2
Welcome to GCOS setup

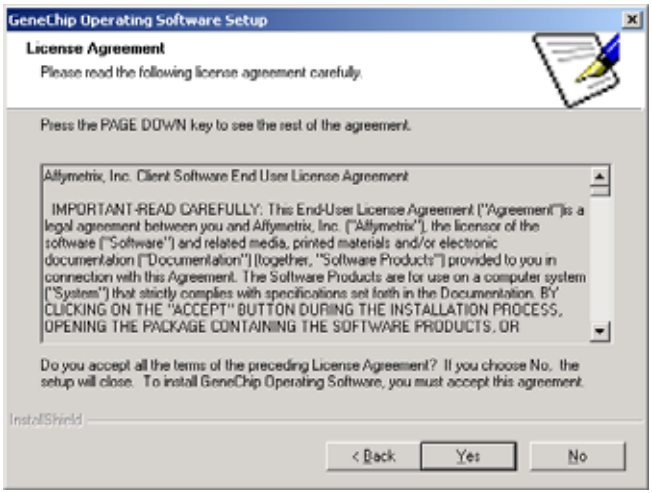


Figure 2.3
License Agreement Window

5. Review the contents and click **Yes** to accept the terms of the licensing agreement.

   ⇒ The Customer Information window appears **(Figure 2.4)**.

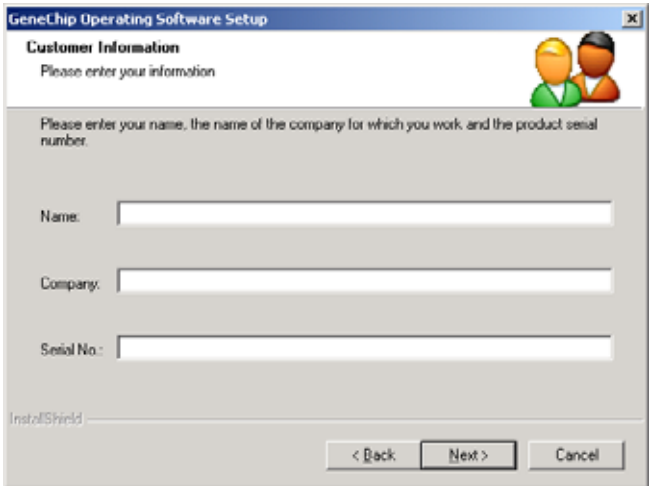


Figure 2.4
Customer Information window

6. Enter your **Name**, **Company**, and **Serial Number**.

   The serial number is located on the Affymetrix® Software Product Registration card.

Note *If you do not have a serial number, contact Affymetrix Technical Support.*

7. Click **Next**.

   ⇒ The GCOS Server Name window appears **(Figure 2.5)**.

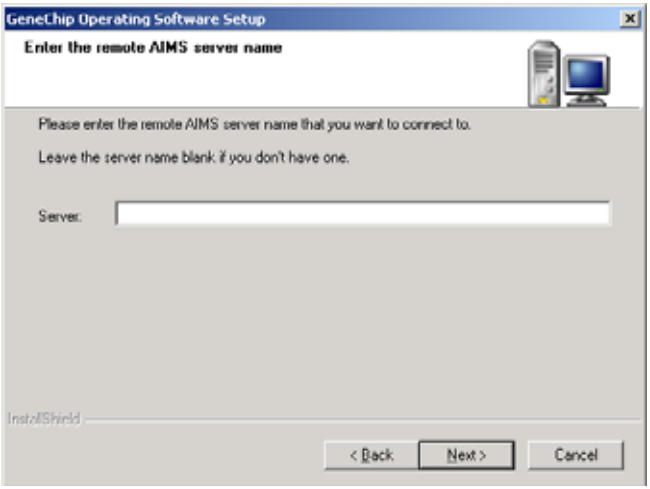


Figure 2.5
GCOS Server Name window

8. Enter the name of the remote GCOS server that you want to connect to. If you do not have one, leave the server name blank.

9. Click **Next**.

   ⇒ The Choose Destination Locations window opens **(Figure 2.6)**.

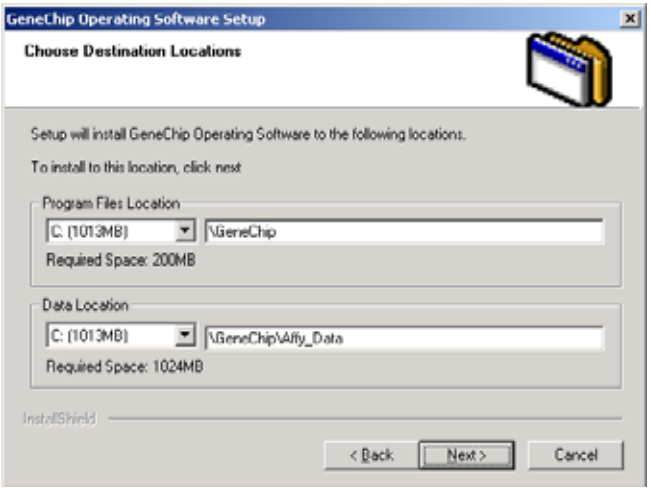


Figure 2.6
Choose Destinations Locations window

10. Select the destination where GCOS will be installed.

    C:\GeneChip is the default location. If you are upgrading from Microarray Suite, we recommend installing in the same directory.

11. Enter and confirm the password for the MSDE system administrator (sa).

✔ Note *The password should be at least six characters long and must contain at least two digits.*

⇒ The installation begins. When the install is completed, the GCOS Software Setup Completed window appears **(Figure 2.7)**.

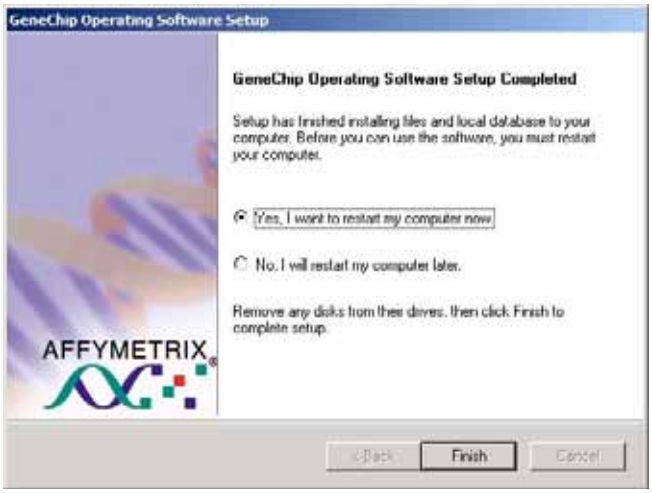


Figure 2.7
GeneChip Operating Software Setup Completed window

12. Choose **Yes, I want to restart my computer now**, and click **Finish**.

    ⇒ The system reboots.

    If you are not prompted to reboot your computer, the installation is complete.

✔ Note

If you are upgrading from a version of Microarray Suite, it is necessary to run the library migrate program to migrate the library files into the local database.

If GCOS will be used with an GCOS server, it is important to ensure the library migration has been completed on the GCOS server.

Steps to Install GCOS for Users Upgrading from Microarray Suite 5.x

Note

You must be logged in as an administrator to install GCOS.

The screen captures depicted in this manual may not exactly match the windows displayed on your screen.

At least 500 MB of available disk space is recommended for the installation.

1. Insert the GCOS CD-ROM.
2. If the autorun feature does not start the program:
   a. Click **Start** →**Run**.
   b. Type *<cd drive letter>:\setup.exe*.
   c. Click **OK**.
   ⇒ The Affymetrix Software Setup window appears **(Figure 2.1)**.

Figure 2.8
Install window

3. Click **Install GCOS**.

4. In the Welcome window **(Figure 2.2)**, click **Next**.

   ⇒ The License Agreement windows appear **(Figure 2.3)**.

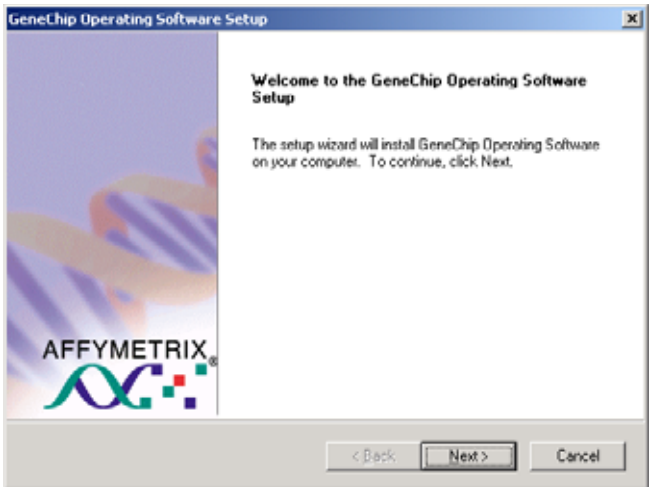


Figure 2.9
Welcome to GCOS setup

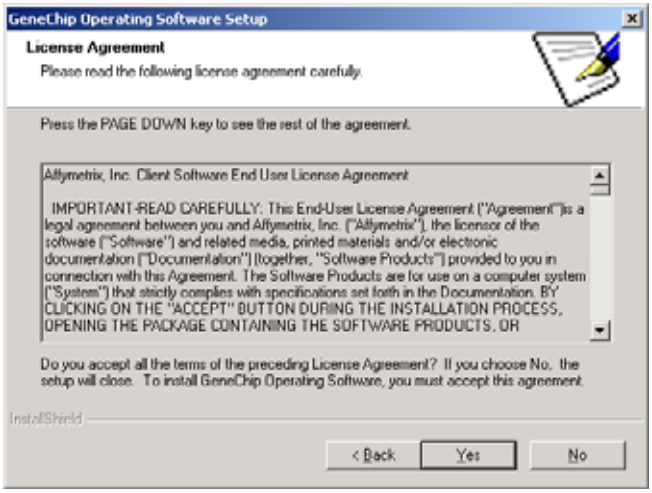


Figure 2.10
License Agreement Window

5. Review the contents and click **Yes** to accept the terms of the licensing agreement.

   ⇒ The Customer Information window appears **(Figure 2.4)**.

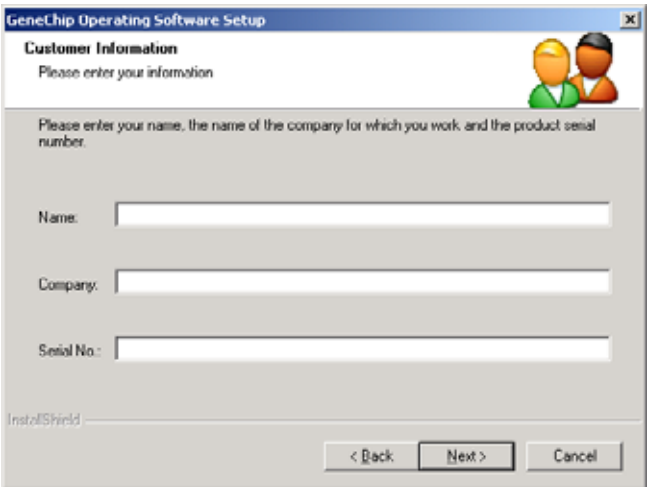


Figure 2.11
Customer Information window

6. Enter your **Name**, **Company**, and **Serial Number**.

   The serial number is located on the Affymetrix® Software Product Registration card.

✔ Note *If you do not have a serial number, contact Affymetrix Technical Support.*

7. Click **Next**.

   ⇒ The GCOS Server Name window appears **(Figure 2.5)**.

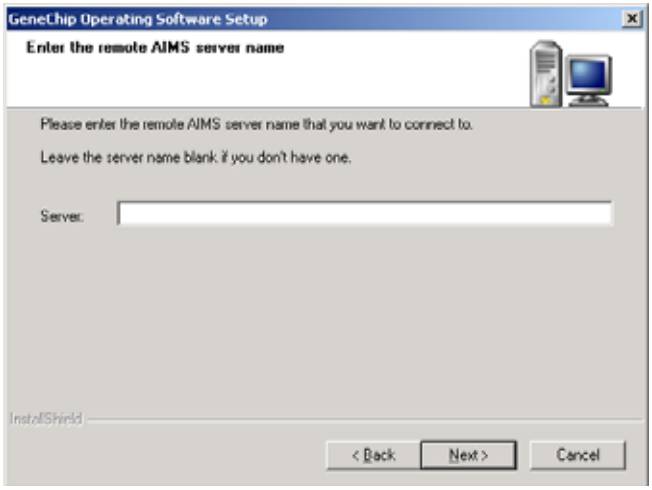


Figure 2.12
GCOS Server Name window

8. Enter the name of the remote GCOS server that you want to connect to. If you do not have one, leave the server name blank.

9. Click **Next**.

   ⇒ The Choose Destination Locations window opens **(Figure 2.6)**.

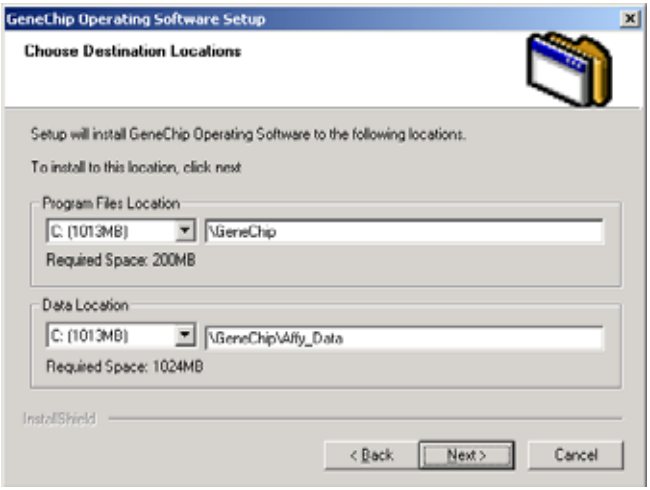


Figure 2.13
Choose Destinations Locations window

10. Select the destination where GCOS will be installed.

    C:\GeneChip is the default location. If you are upgrading from Microarray Suite, we recommend installing in the same directory.

11. Enter and confirm the password for the MSDE system administrator (sa).

Note

The password should be at least six characters long and must contain at least two digits.

⇒ The software prompts you to start the batch import tool after the installation is complete **(Figure 2.14)**.

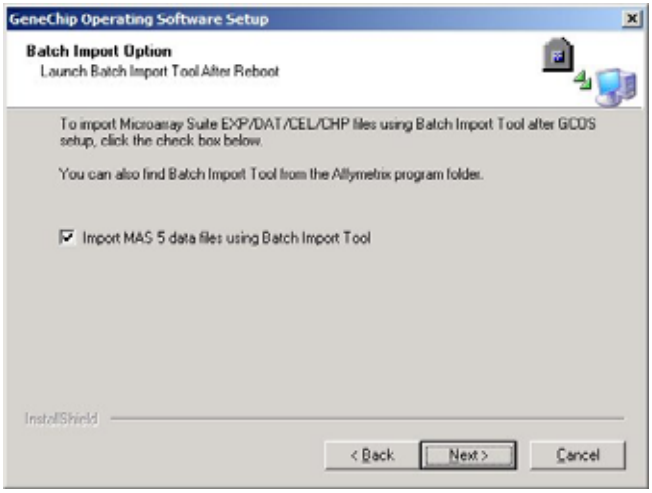


Figure 2.14
Batch Import Option window

12. To import Microarray Suite files (.exp, .dat, .cel, and .chp) after the installation is complete, place a check mark next to **Import MAS 5 data files using Batch Import Tool.**

    If you do not select batch import option at this time, you can access the batch import tool later from the Windows Start menu (**Start** → **Programs** →**Affymetrix** →**Batch Import Tool**).

13. Click **Next**.

    ⇒ The installation begins. When the install is completed, the GeneChip Operating Software Setup Completed window appears **(Figure 2.15)**.

Figure 2.15
GeneChip Operating Software Setup Completed

14. Choose **Yes, I want to restart my computer now** and click **Finish**.

    ⇒ The system reboots.

15. After the reboot, login using the same user name.

✔ Note

To complete the installation, you must reboot the computer and login again as the same user.

    ⇒ The library update program starts.

16. To install library files, refer to *Steps for Installing Library Files on an Upgraded GCOS Install,* on page 30.

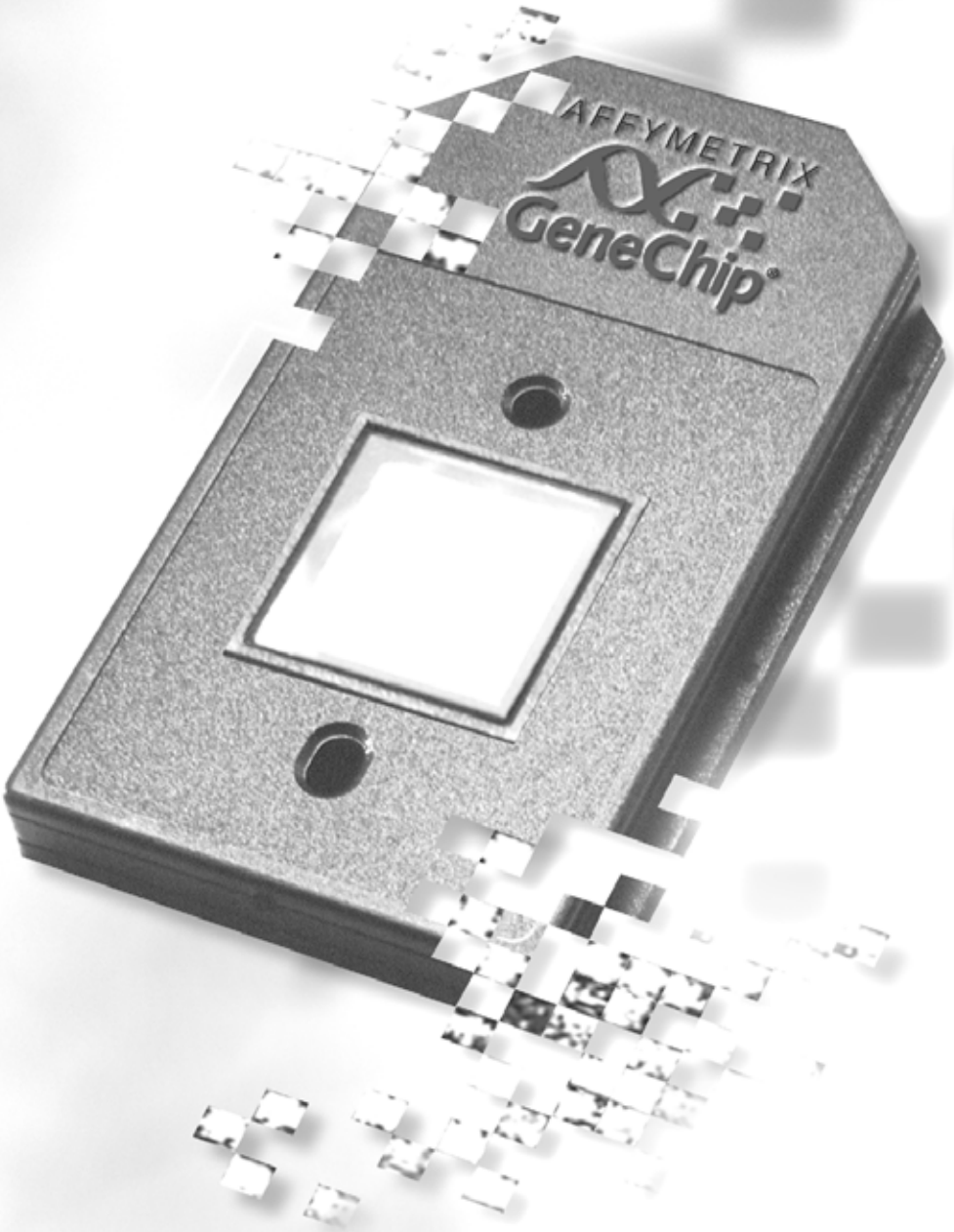
    To run the library update program at another time, navigate to the GeneChip\Migrate directory and run the setup.exe.

✔ Note

If you are upgrading from a version of Microarray Suite, it is necessary to run the library migrate program to migrate the library files. Alternatively, use GCOS to scan and analyze the data.

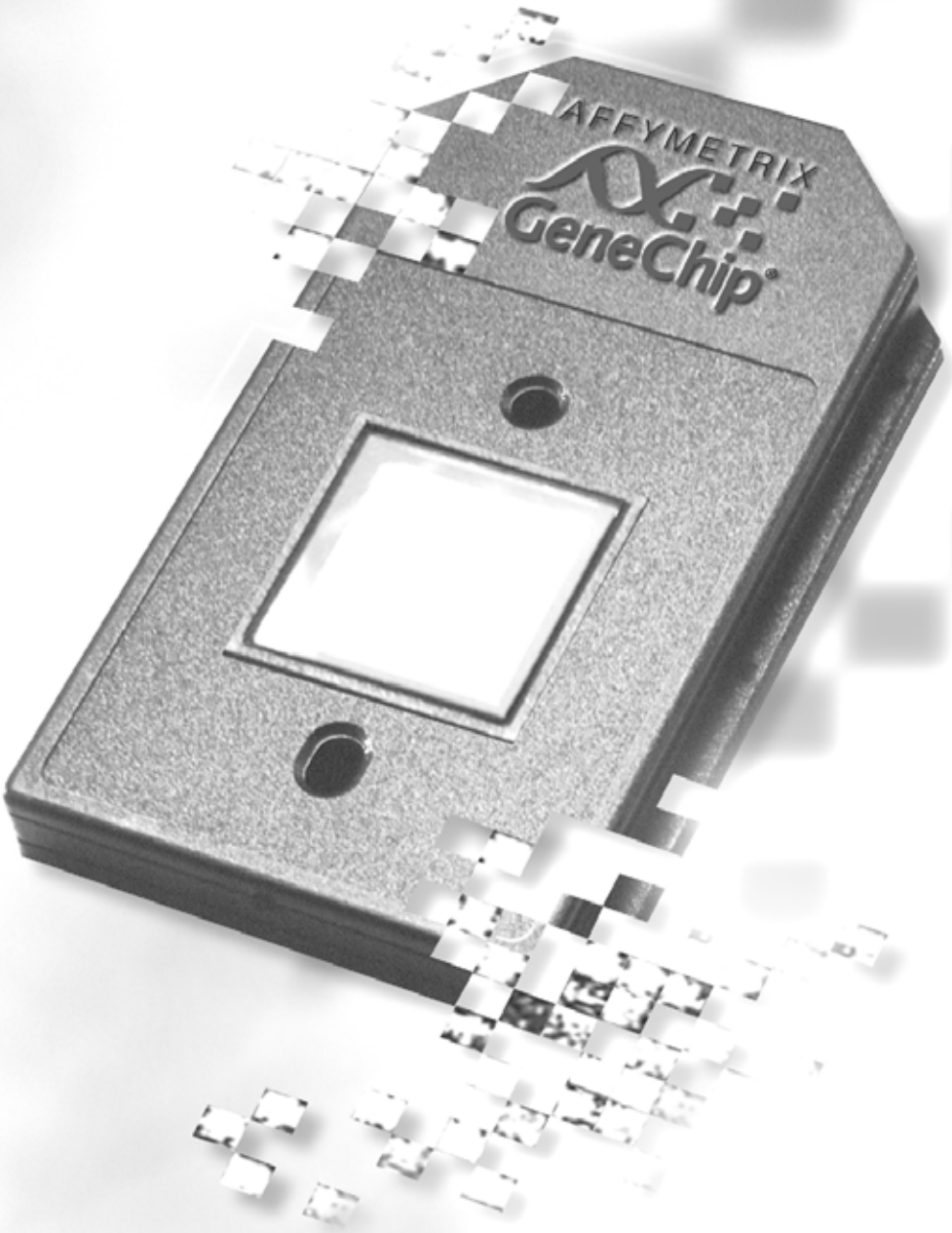
17. When the library update program is completed, the batch import tool starts (if this option was selected in step 12).

To import Microarray Suite files to GCOS, refer to the instructions for the batch import tool on the GCOS CD. This completes the install.



3

Chapter 3



Affymetrix® GeneChip® Library Install

This chapter provides detailed instructions for installing Affymetrix® GeneChip® probe array library files using the library install program. The probe information or library data include the probe array design characteristics, probe utilization and content, and scanning and analysis parameters. Library data are unique to each probe array type. The library install program includes a full install and an update program. The full install should be used when installing the Affymetrix® GeneChip® Operating Software (GCOS) on a new system. If upgrading from a previous version of Affymetrix® Microarray Suite, use the library update program.

Steps for Installing Library Files on a New GCOS Install

This section of the manual guides you through the full library install program. The full install is required on a GCOS system that was not upgraded from a previous version of Affymetrix® Microarray Suite.

1. Insert the library files CD-ROM for the appropriate probe array type.

   ⇒ The Affymetrix Library Files Setup window appears **(Figure 3.1)**.

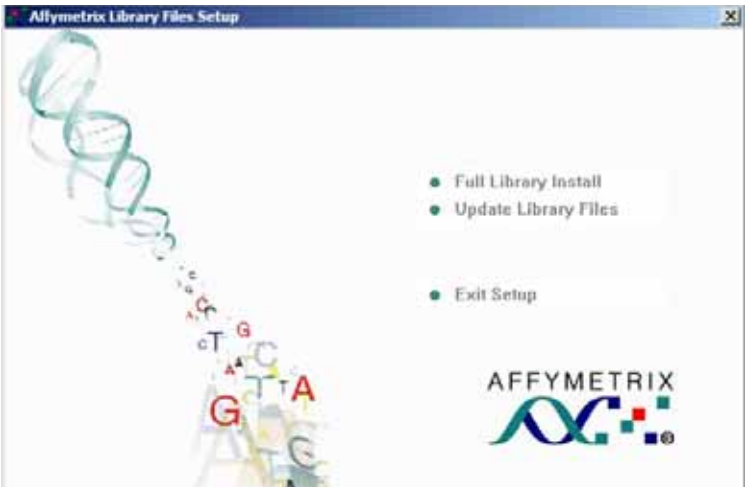


Figure 3.1
Library Files Setup window

2. Click **Full Library Install**.

   ⇒ The InstallShield Wizard for Affymetrix Library Files starts **(Figure 3.2)**.

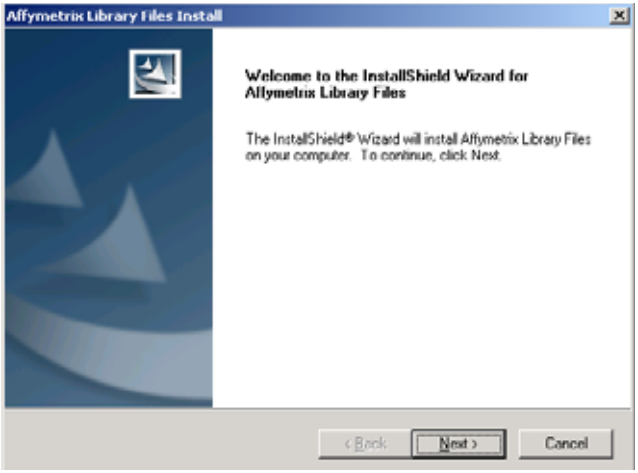


Figure 3.2
InstallShield Wizard Welcome window

✔ Note

GCOS does not allow you to modify the location of the library file directory. By default, all probe array type information is installed in the library subdirectory of the GCOS installation.

Do not move or delete the library directory or the files in the directory. *This will render the GCOS installation invalid.*

3. Click **Next**.

   ⇒ The Select Probe Array Types window appears **(Figure 3.3)**.

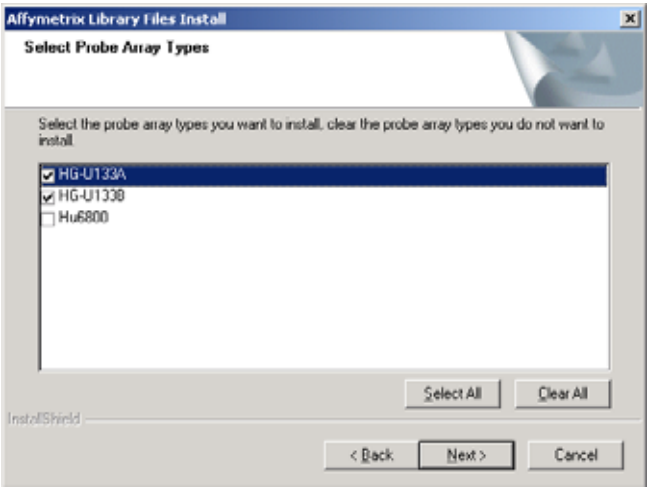


Figure 3.3
Select Probe Array Types window

4. Click **Select All** to select all array types for installation. Alternatively, place a check mark next to the array type(s) that you want to install.

   For example, in **Figure 3.3**, the HG-U133A and HG-U133B probe arrays will be installed on the system.

5. Click **Next**.

   ⇒ The Start Installing Files window appears **Figure 3.4**.

   This window shows the install options selected, including the:

   - probe array types that will be installed on the system
   - location of the library directory where the probe array library data will be saved
   - location of the protocol directory where the fluidic station script files will be saved.

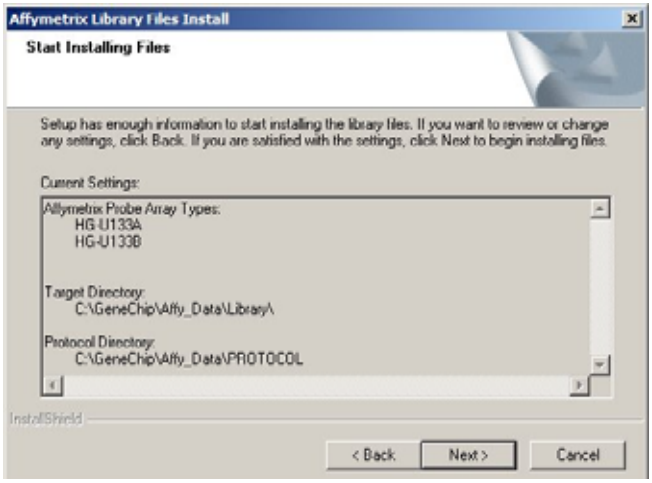


Figure 3.4
Start Installing Files window

6. Click **Next**.

   ⇒ The install process begins.

   After the installation is complete, the software displays the Finish window **(Figure 3.5)**.

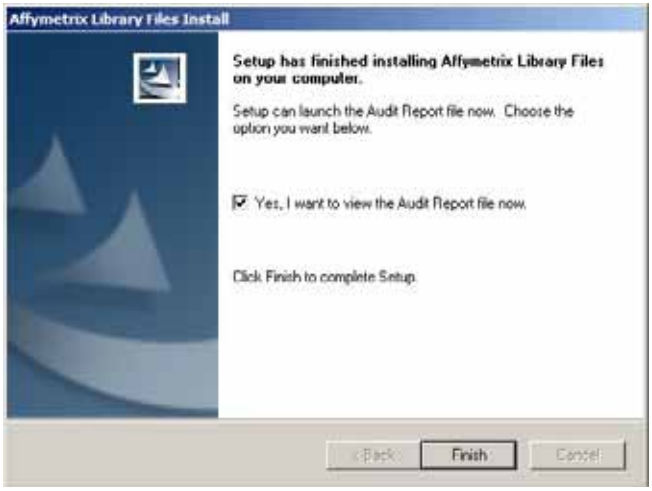


Figure 3.5
Finish window

7. Choose **Yes, I want to view the Audit Report file now**, and click **Finish**.

   ⇒ The installation completes.

   If an error occurs, the software displays an error dialog box **Figure 3.6.**

Figure 3.6
Error dialog box

8. Click **Yes** to view the SQL.log and Audit XXX.log that contain information about the error that occurred. Forward this file to Affymetrix® technical support to obtain a solution for the problem.

Steps for Installing Library Files on an Upgraded GCOS Install

The library install program includes a full install and an update program. Use the update program when you upgrade from a version of Affymetrix® Microarray Suite to GCOS. The update program migrates probe array type information from the files in the library directory that was used with Microarray Suite.

1. Insert the library files CD_ROM for the appropriate probe array type.

   ⇒ The Affymetrix Library Files Setup window appears **(Figure 3.7)**.

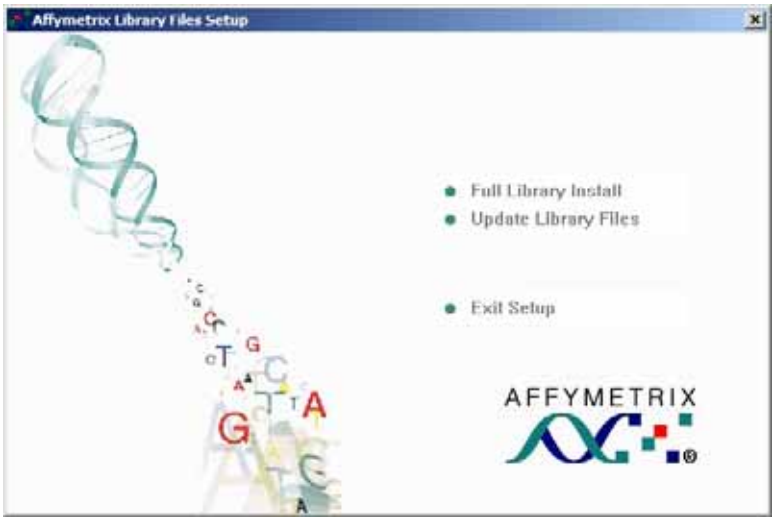


Figure 3.7
Library Files Setup window

2. Click **Update Library Files**.

   ⇒ The InstallShield Wizard for Affymetrix Library Files starts **(Figure 3.8)**.

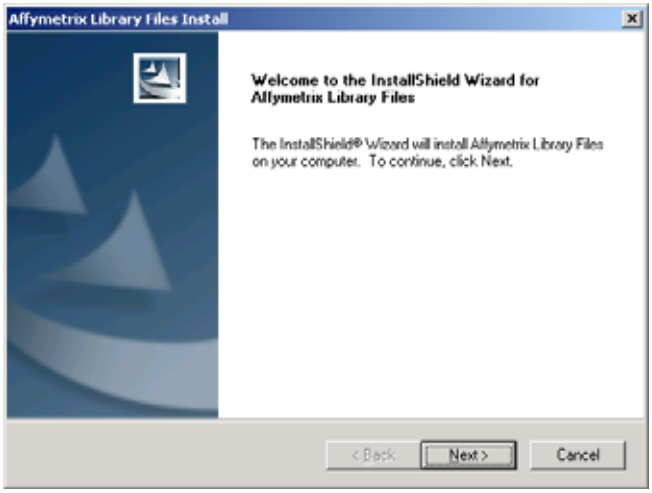


Figure 3.8
InstallShield Wizard Welcome window

3. Click **Next**.

   ⇒ The Select Source Library Directory window appears **(Figure 3.9)**.

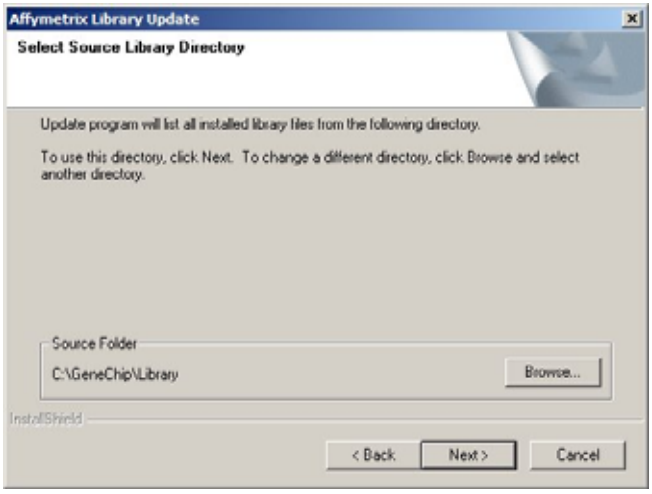


Figure 3.9
Select Source Library Directory window

4. To select the location of the source library directory from which to migrate the information into the GCOS database, click **Browse**. Click **Next**.

Note

This library directory is the directory that was used with Affymetrix Microarray suite. It contains .CIF and .CDF files that contain information about the probe array types used in the system.

⇒ The Select Source Protocol Directory window appears **(Figure 3.10)**.

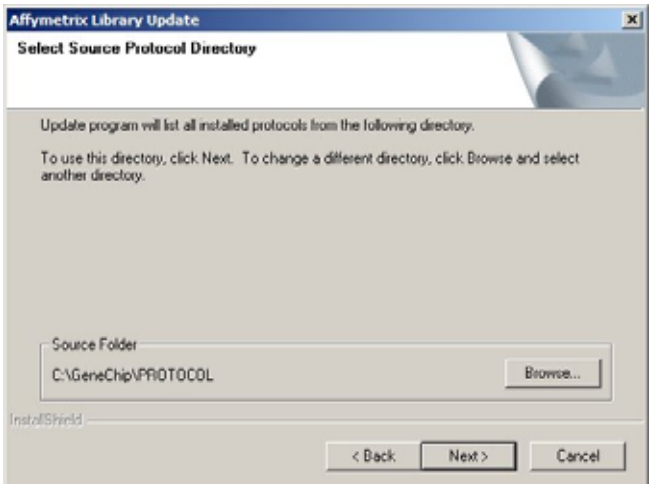


Figure 3.10
Select Source Protocol Directory window

5. Click **Browse** and select the location of the fluidics protocols directory from which to migrate the information into the GCOS database.
6. Click **Next** to display the probe array types available for migration.

   ⇒ The Select Probe Array Types window appears **(Figure 3.11)**.

✔ Note

To migrate a probe array type from a previous version of Microarray Suite software, the library directory must contain the .CIF and .CDF files. The update program will not install the gene descriptions, sequences, or annotations. These should exist in the GCOS database from the previous Microarray Suite install.

To migrate fluidics scripts from the protocols directory, the .BIN file must exist along with the .MAC file. If the .BIN file is missing, the software will not update the database with the associated script. However, if a .BIN file exists in the directory without the associated .MAC file (for example, the "Home" script that can be executed without selecting an experiment) the update program will copy the file to the new protocols directory.

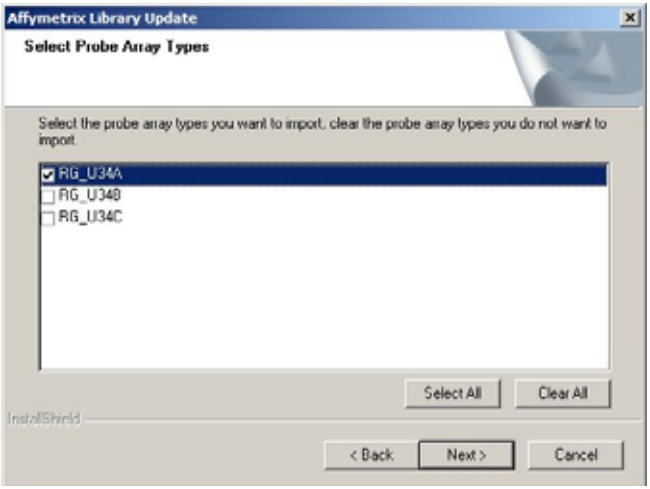


Figure 3.11
Select Probe Array Types window

7. Select the probe array types that you want to migrate from the library directory. Click **Select All** to select all of the array types or place a check mark next to the probe array type(s) that you want to install.

   For example, in **Figure 3.11**, the RG-U34A probe array will be installed on the system.

8. Click **Next**.

   ⇒ The Select Protocols window appears and displays the fluidics protocols that will be migrated to the GCOS installation **Figure 3.12**.

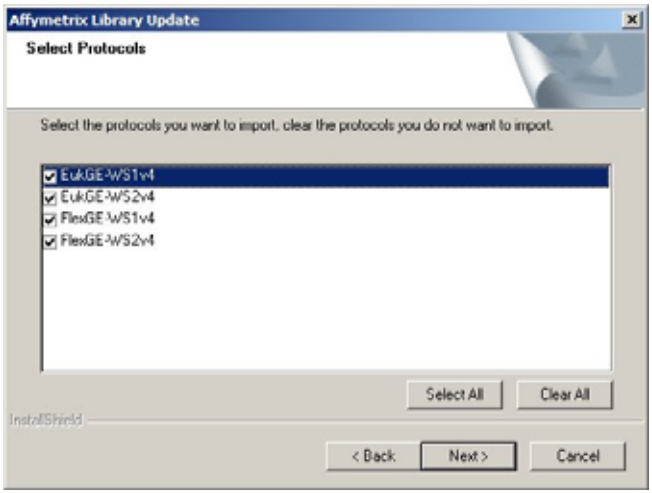


Figure 3.12
Select Protocols window

9. Select the fluidics scripts that you want to migrate from the protocols directory. To select all of the scripts, click **Select All**. To select scripts individually, place a check mark next to the script.

   For example, in **Figure 3.12**, the scripts EukGE-WS1v4, EukGE-WS2v4, FlexGE-WS1v4, FlexGE-WS2v4 will be installed on the system.

10. Click **Next**.

    ⇒ The Start Importing Library Files window appears **(Figure 3.13)**.

    It shows the:

    - probe array types and fluidics scripts that will be installed
    - location of the library directory for the probe array type data
    - location of the protocol directory for the fluidics station scripts

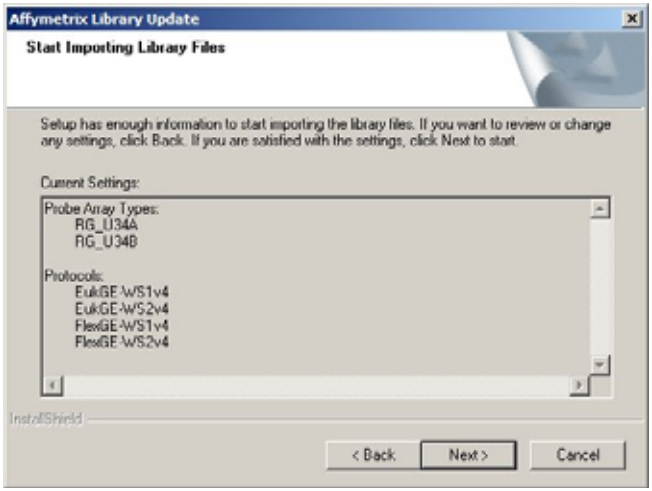


Figure 3.13
Start Importing Library Files window

11. Click **Next** to start the installation.

    After the installation is complete, the software displays the Finish window **(Figure 3.14)**.

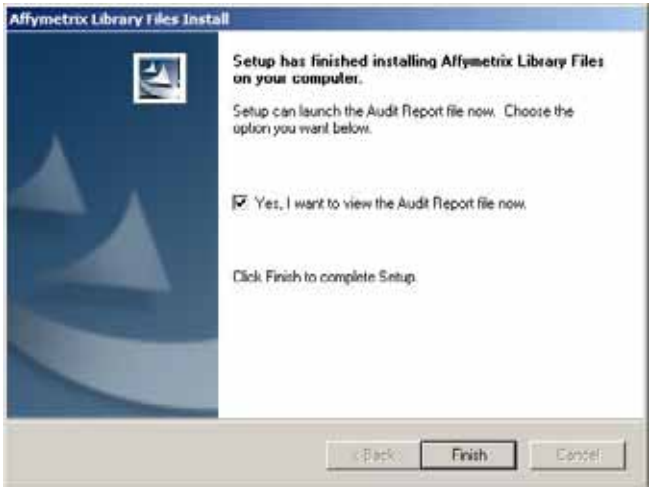


Figure 3.14
Finish window

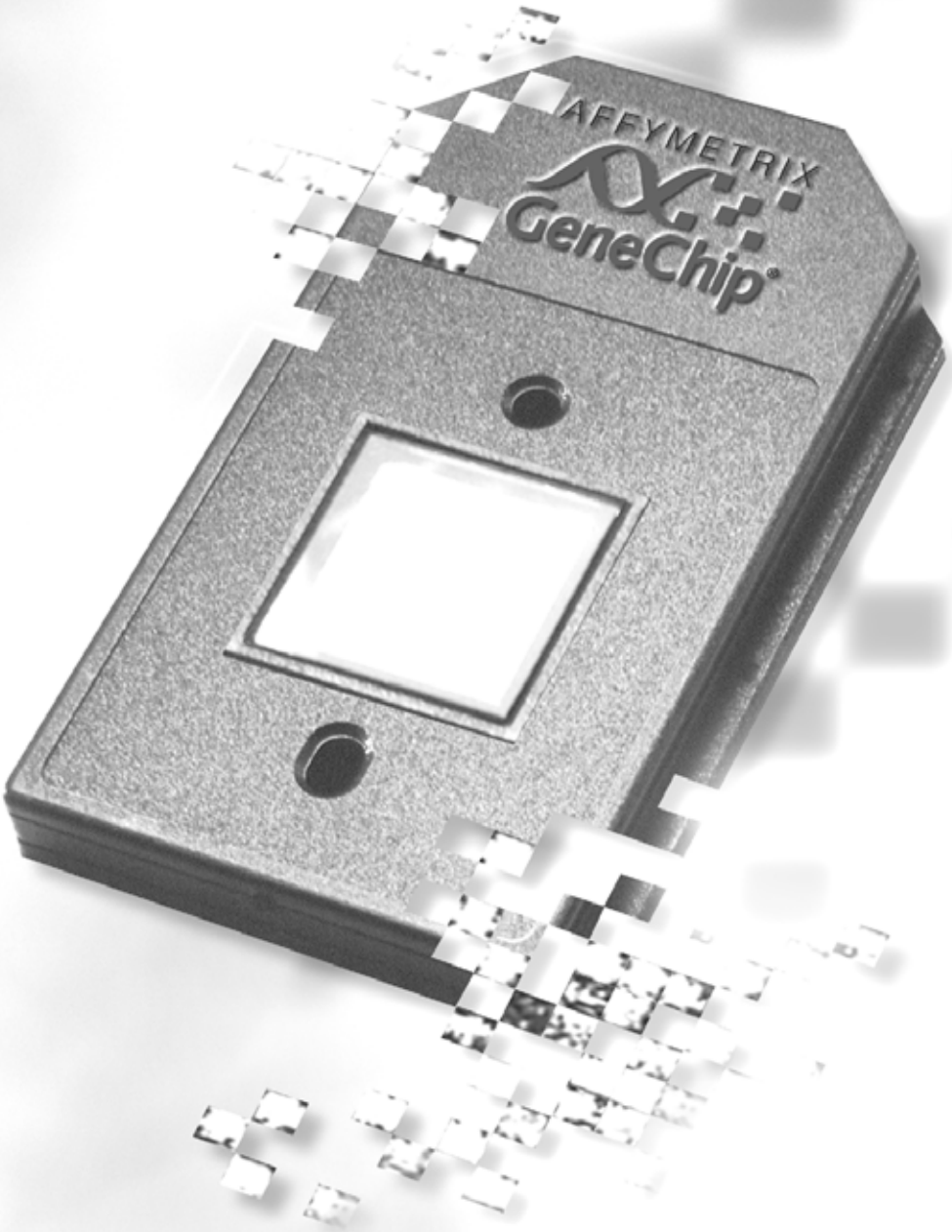
12. Choose **Yes, I want to view the Audit Report file now** and click **Finish**.

    ⇒ The installation completes.

    If an error occurs, the software displays an error dialog box **(Figure 3.15)**.

Figure 3.15
Error dialog box

13. Click **Yes** to view the sql.log and updateXXX.log files that contain information about the error that occurred. Forward this file to Affymetrix® technical support to obtain a solution for the problem.



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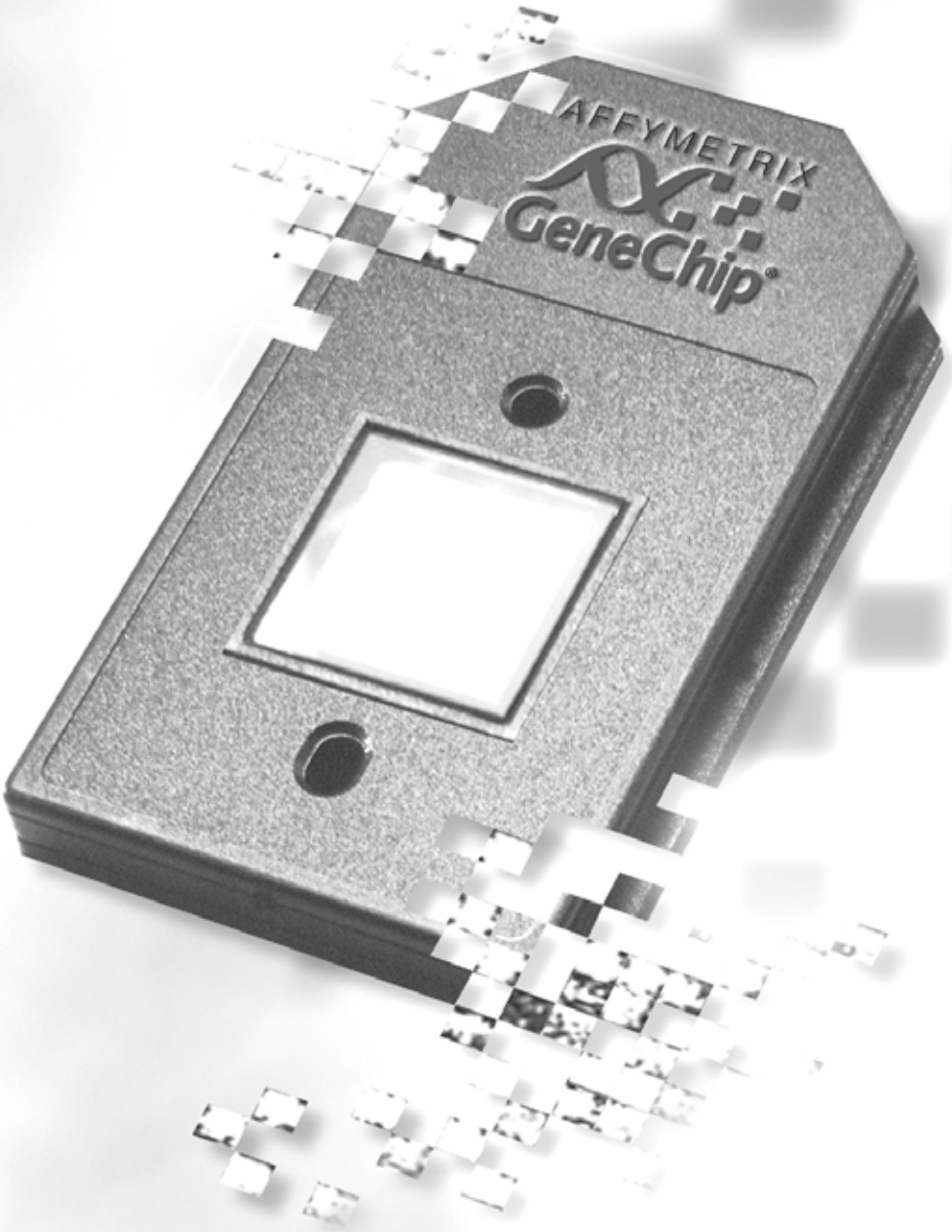
Chapter 4



GeneChip® System Overview

This chapter provides background information on the Affymetrix® GeneChip® array and instrument platform. It provides a brief overview of the software applications that acquire, analyze, and manage the data.

System Overview

The Affymetrix® GeneChip® platform uses high-density GeneChip oligonucleotide probe arrays to efficiently acquire and analyze genetic information. This integrated and complete system includes the:

- GeneChip® Hybridization Oven 640
- GeneChip® Fluidics Station
- Affymetrix® GeneChip® Scanner 3000 or the GeneArray® 2500 scanner
- Computer workstation
- GeneChip® Operating Software (GCOS) 1.0 (formerly Microarray Suite)
- GCOS Manager (formerly LIMS Manager)
- GCOS Administrator
- GeneChip® Operating Software Server (GCOS Server) (formerly LIMS)
- Affymetrix® Data Mining Tool (DMT)
- GeneChip® DNA Analysis Software (GDAS)

GeneChip® Assay

The GeneChip® Operating Software (GCOS) automates instrument control and provides a central infrastructure to acquire, track, and analyze experiment data. **Table 4.1** shows the major steps and software functions in a GeneChip® assay.

Table 4.1
GeneChip® Assay

Assay Step	Output	Software Function
Prepare the target.		
Register the sample and set up an experiment.	Experiment information	GCOS adds the sample and experiment information to the process database. GCOS tracks the sample and the associated experiment data (image, cell intensities, and probe analysis data) in the workflow monitor.
Hybridize, wash, and stain the probe array.		GCOS automates control of the fluidics station and scanner.
Scan the probe array.	Image data	GCOS automates control of the scanner, acquires and saves the scan data.
	Cell intensity data	GCOS automatically computes and saves the cell intensities from the image data. GCOS tracks the image and cell intensity data in the process database.
Analyze the cell intensities.	Probe analysis	The GCOS Statistical Expression algorithm analyzes the cell intensities of expression arrays, and saves the probe analysis or chip data. The GDAS algorithms analyze the cell intensities of other types of GeneChip® arrays, and saves the probe analysis or *chip* data.
Generate a report.	Report (.rpt)	GCOS generates and saves the Expression report (.rpt). GDAS generates and saves a report (.rpt) for array types other than expression.
Publish the sample and experiment data.		GCOS copies expression experiment data (cell intensity and probe analysis data) to the publish database where the data are accessible to the Affymetrix® Data Mining Tool (DMT) software and other third party analysis tools.

GCOS provides a workflow-based tracking system for the data. The workflow monitor **(Figure 4.1)** tracks sample or experiment status through a series of steps or queue that includes:

- hybridization
- scan
- grid alignment
- cell intensity analysis (for image data files that require manual grid alignment)
- probe array analysis for expression assay
- publish expression probe array analysis data

You can easily confirm the status of an experiment by checking the workflow monitor. For example, the Hybridization tab shows experiments to be processed in the fluidics station.

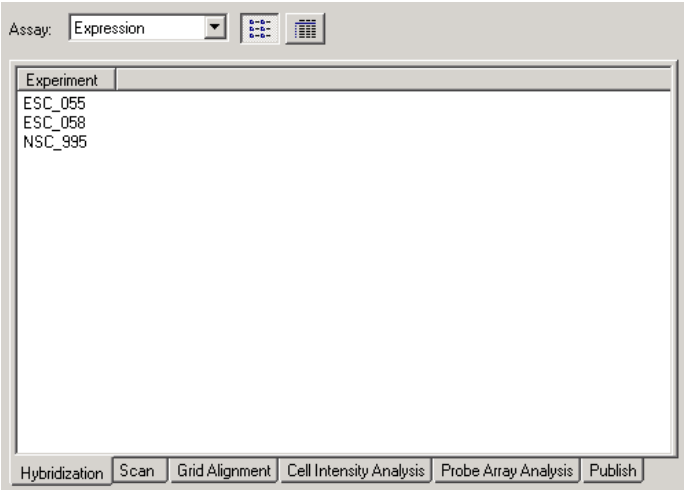


Figure 4.1
Workflow monitor, Hybridization tab

Preparing the Target Sample

Refer to the appropriate GeneChip® probe array package insert, the *Expression Analysis Technical Manual*, or to other Affymetrix® technical documentation relevant to your particular project.

Registering a Sample and Setting Up an Experiment

You must identify or *register* a sample (target) and define an experiment in GCOS before processing a probe array in the fluidics station or scanning an array. You register the sample by entering the sample type (required) and sample information (*attributes*), and associating the sample with a project (which may include several different samples) **(Figure 4.2)**. The registration process adds the sample information to the process database. Now GCOS tracks the sample and the associated experiment data (image, cell intensities, probe analysis data) in the workflow monitor.

To define an experiment, you specify the type of probe array to which the target sample will be hybridized as well as other information relevant to the experiment **(Figure 4.2)**. You can also associate a barcode with an experiment. If an Affymetrix® barcode is entered (manually or using a keyboard wedge reader), GCOS automatically reads and displays the probe array type, lot number, and expiration date. GCOS uses the experiment information to select scanner specifications for the probe array and identify the user-modifiable algorithm parameters for data analysis.

You can also use a template (defined in GCOS Manager) to register a sample and define an experiment. For more information on creating a template, see *Template Tab*, on page 344.

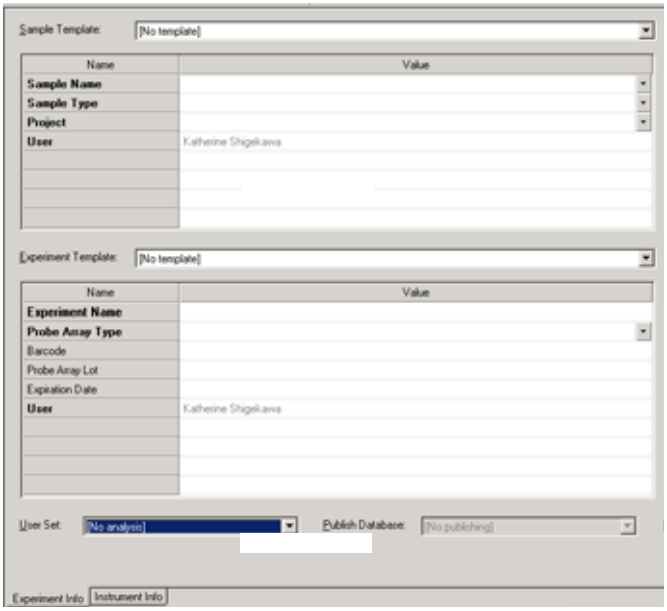


Figure 4.2
Sample and experiment information

Hybridizing, Washing, and Staining a Probe Array

During hybridization, the probe array is incubated with a hybridization cocktail containing the labeled target and control oligonucleotides in a buffer optimized for each type of probe array (refer to the appropriate GeneChip® probe array package insert).

The hybridization reaction occurs in the GeneChip® fluidics station, except for assays requiring longer hybridization periods in which case the hybridization reaction occurs in the GeneChip® Hybridization Oven 640.

During hybridization in the fluidics station, the probe array is repeatedly filled and drained with the hybridization cocktail. The strongest hybridization occurs between the array probes and sequences in the target that are most nearly complementary.

After hybridization, the probe array undergoes a series of stringent washes (specifically optimized for each type of probe array) in the fluidics station. If

the target was labeled with a fluorescent tag, the array may be scanned at this point using the GeneChip® Scanner 3000 or the GeneArray® scanner. In applications that use a biotin-labeled target, the probe array must be stained with a streptavidin-conjugated fluorescent stain and requires antibody amplification prior to scanning.

GCOS controls the fluidics station using pre-programmed fluidics protocols for hybridizing, washing, and staining the probe arrays. The computer workstation running GCOS can simultaneously control up to eight fluidics stations. A fluidics station contains four modules and each module can independently process a probe array using different fluidics protocols. Some of the fluidics protocols may be customized. Utilities for station maintenance are also included.

Scanning a Probe Array

After the GeneChip® probe array has undergone hybridization, washing, and staining, it is ready to be scanned.

GCOS automates control of the scanner and uses the experiment information to manage the scanner settings for each type of probe array. The software enables you to start a scan and view the intensity data as it is collected during scanning. After the scan is completed, GCOS displays a picture of the image data in the Image window **(Figure 4.3)**. GCOS tracks the image data and the scan parameters in the process database. For more information on image data, see Chapter 9, *Working with Images* on page 135.

The Affymetrix® GeneChip® Scanner 3000 requires about 4 to 7 minutes to scan an array depending on the size of the scan region, the number of scans, the autofocusing adjustment, and other parameters.

✔ Note *The Affymetrix® Scanner 3000 cannot scan fluorescein labeled arrays.*

The GeneArray® 2500 scanner requires 5 to 25 minutes to scan an array depending on the size of the scan region, the number of scans, and other parameters.

Computing Cell Intensities

After the scan is completed, GCOS displays a picture of the scan image in the image window. The software represents the fluorescence intensity values

from each pixel on the array in a grayscale or pseudocolor mode and superimposes a grid on the image to delineate the probe cells (features).

GCOS automatically:

- analyzes the image data and computes a single intensity value for each probe cell on an array
- saves the cell intensity data **(Figure 4.3)**

GCOS tracks the cell intensity data and the parameters used in the cell intensity analysis in the process database.

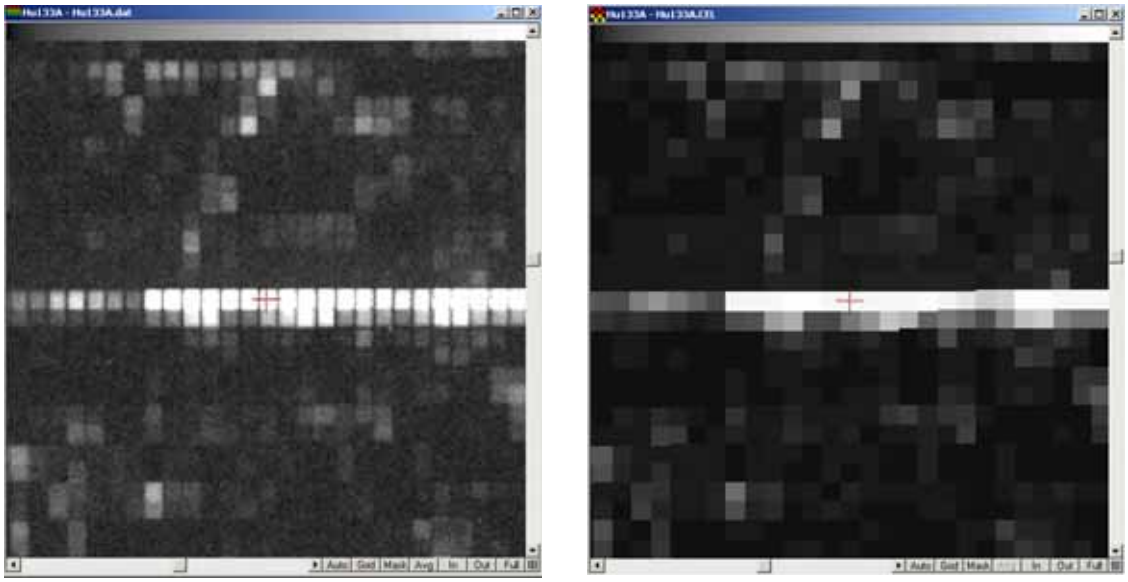


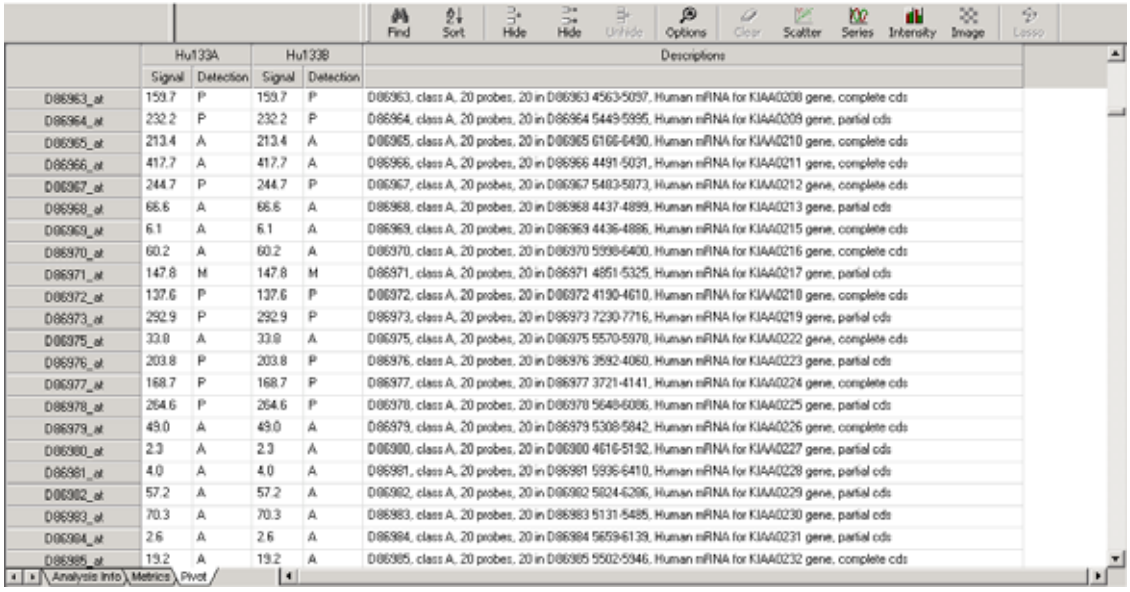
Figure 4.3
Image window, scan image (left) and computed cell intensity data (right)

Expression Analysis

The Statistical Expression algorithm processes the cell intensity data. A single-array analysis examines the cell intensity data from one experiment (GeneChip® probe array). For each transcript represented on the array, the algorithm computes the detection call (present, absent, or marginal (unable to call the transcript present or absent), or no call) and other call metrics.

A comparison analysis compares the cell intensities of the same probe set on two different probe arrays (an experiment and baseline of the same probe array type) to determine the relative change in the expression level of a transcript. For each transcript represented on the array, the algorithm computes the change call (increase, marginal increase, no change, marginal decrease, decrease) or no call, and other call metrics.

GCOS saves the probe analysis data and automatically displays it in the Expression Analysis window **(Figure 4.4)**. GCOS tracks the probe analysis data and the analysis parameters in the process database.



	Hu133A		Hu133B		Descriptions
	Signal	Detection	Signal	Detection	
D86963_at	159.7	P	159.7	P	D86963, class A, 20 probes, 20 in D86963 4563-5037, Human mRNA for KIAA0208 gene, complete cds
D86964_at	232.2	P	232.2	P	D86964, class A, 20 probes, 20 in D86964 5449-5995, Human mRNA for KIAA0209 gene, partial cds
D86965_at	213.4	A	213.4	A	D86965, class A, 20 probes, 20 in D86965 6166-6490, Human mRNA for KIAA0210 gene, complete cds
D86966_at	417.7	A	417.7	A	D86966, class A, 20 probes, 20 in D86966 4491-5031, Human mRNA for KIAA0211 gene, complete cds
D86967_at	244.7	P	244.7	P	D86967, class A, 20 probes, 20 in D86967 5483-5973, Human mRNA for KIAA0212 gene, complete cds
D86968_at	66.6	A	66.6	A	D86968, class A, 20 probes, 20 in D86968 4437-4899, Human mRNA for KIAA0213 gene, partial cds
D86969_at	6.1	A	6.1	A	D86969, class A, 20 probes, 20 in D86969 4436-4886, Human mRNA for KIAA0215 gene, complete cds
D86970_at	60.2	A	60.2	A	D86970, class A, 20 probes, 20 in D86970 5998-6400, Human mRNA for KIAA0216 gene, complete cds
D86971_at	147.8	M	147.8	M	D86971, class A, 20 probes, 20 in D86971 4851-5325, Human mRNA for KIAA0217 gene, partial cds
D86972_at	137.6	P	137.6	P	D86972, class A, 20 probes, 20 in D86972 4190-4610, Human mRNA for KIAA0218 gene, complete cds
D86973_at	292.9	P	292.9	P	D86973, class A, 20 probes, 20 in D86973 7230-7716, Human mRNA for KIAA0219 gene, partial cds
D86975_at	33.8	A	33.8	A	D86975, class A, 20 probes, 20 in D86975 5570-5978, Human mRNA for KIAA0222 gene, complete cds
D86976_at	203.8	P	203.8	P	D86976, class A, 20 probes, 20 in D86976 3592-4060, Human mRNA for KIAA0223 gene, partial cds
D86977_at	168.7	P	168.7	P	D86977, class A, 20 probes, 20 in D86977 3721-4141, Human mRNA for KIAA0224 gene, complete cds
D86978_at	264.6	P	264.6	P	D86978, class A, 20 probes, 20 in D86978 5648-6086, Human mRNA for KIAA0225 gene, partial cds
D86979_at	49.0	A	49.0	A	D86979, class A, 20 probes, 20 in D86979 5308-5842, Human mRNA for KIAA0226 gene, complete cds
D86980_at	2.3	A	2.3	A	D86980, class A, 20 probes, 20 in D86980 4616-5192, Human mRNA for KIAA0227 gene, partial cds
D86981_at	4.0	A	4.0	A	D86981, class A, 20 probes, 20 in D86981 5936-6410, Human mRNA for KIAA0228 gene, partial cds
D86982_at	57.2	A	57.2	A	D86982, class A, 20 probes, 20 in D86982 5824-6286, Human mRNA for KIAA0229 gene, partial cds
D86983_at	70.3	A	70.3	A	D86983, class A, 20 probes, 20 in D86983 5131-5485, Human mRNA for KIAA0230 gene, partial cds
D86984_at	2.6	A	2.6	A	D86984, class A, 20 probes, 20 in D86984 5659-6139, Human mRNA for KIAA0231 gene, partial cds
D86985_at	19.2	A	19.2	A	D86985, class A, 20 probes, 20 in D86985 5502-5946, Human mRNA for KIAA0232 gene, complete cds

Figure 4.4
Expression Analysis window, probe analysis data

Note *GCOS analyzes expression cell intensity data only. GDAS analyzes cell intensity data from other types of probe arrays.*

Publishing Experiment Data

GCOS copies or *publishes* expression experiment data (cell intensity and probe analysis data) in AADM format to the publish database (see **Figure 4.5** on page 49). The publish database is accessible to the Affymetrix® Data Mining Tool (DMT) or several other third party analysis tools. The DMT is a separate application that provides powerful query and analysis tools.

Databases

GCOS installs Microsoft® Data Engine (MSDE) databases on the workstation. **Table 4.2** shows the GCOS databases and their functions. Alternatively, the Affymetrix® GeneChip® Operating Software Server (GCOS Server) installs Microsoft® SQL Server or Oracle databases on a network accessible server. GCOS also provides the end user interface to GCOS Server. GCOS Server enables users to register samples and set up experiments, and access and analyze data from any client workstation on the network.

Table 4.2
GCOS databases

Database Name	Contents
Process	Data tracking information about samples and experiments, and information about the image, intensity, and probe analysis data for a particular sample and experiment.
Publish	Expression data in the Affymetrix® Analysis Data Model (AADM) database schema that can be accessed by the Affymetrix® Data Mining Tool (DMT) and other software.
Gene Information	Accession numbers, probe set descriptions, target sequence, and user annotations accessible to DMT.

Database Management & Administration

GCOS Manager is a separate application that helps you manage your expression data. This software tool provides a convenient way to:

- manage the process and publish databases
- delete process or publish data
- create templates for entering sample and experiment attributes
- create a userset (a predefined set of Statistical Expression algorithm parameter settings) for expression analysis
- archive image, cell intensity, and probe analysis data to a network drive
- restore image, cell intensity, and probe analysis data to a network drive
- export an experiment and the associated data files to a user-specified location on the workstation
- define user privileges (roles) for the GCOS server
- apply additional file security to the GCOS server

GCOS Administrator enables you to:

- monitor free and used workstation disk space
- back up a publish or process database, sample, or project on the workstation to an archival file (.cab) that can be restored to another GCOS system
- restore a publish or process database, sample, or project from an archival file (.cab) to another GCOS system
- change the location of a publish database
- change the password for the process database or a publish database

Together GCOS, GCOS Manager, and GCOS Administrator provide full functionality for the GeneChip® instrument and array platform as well as basic expression analysis **(Figure 4.5)**.

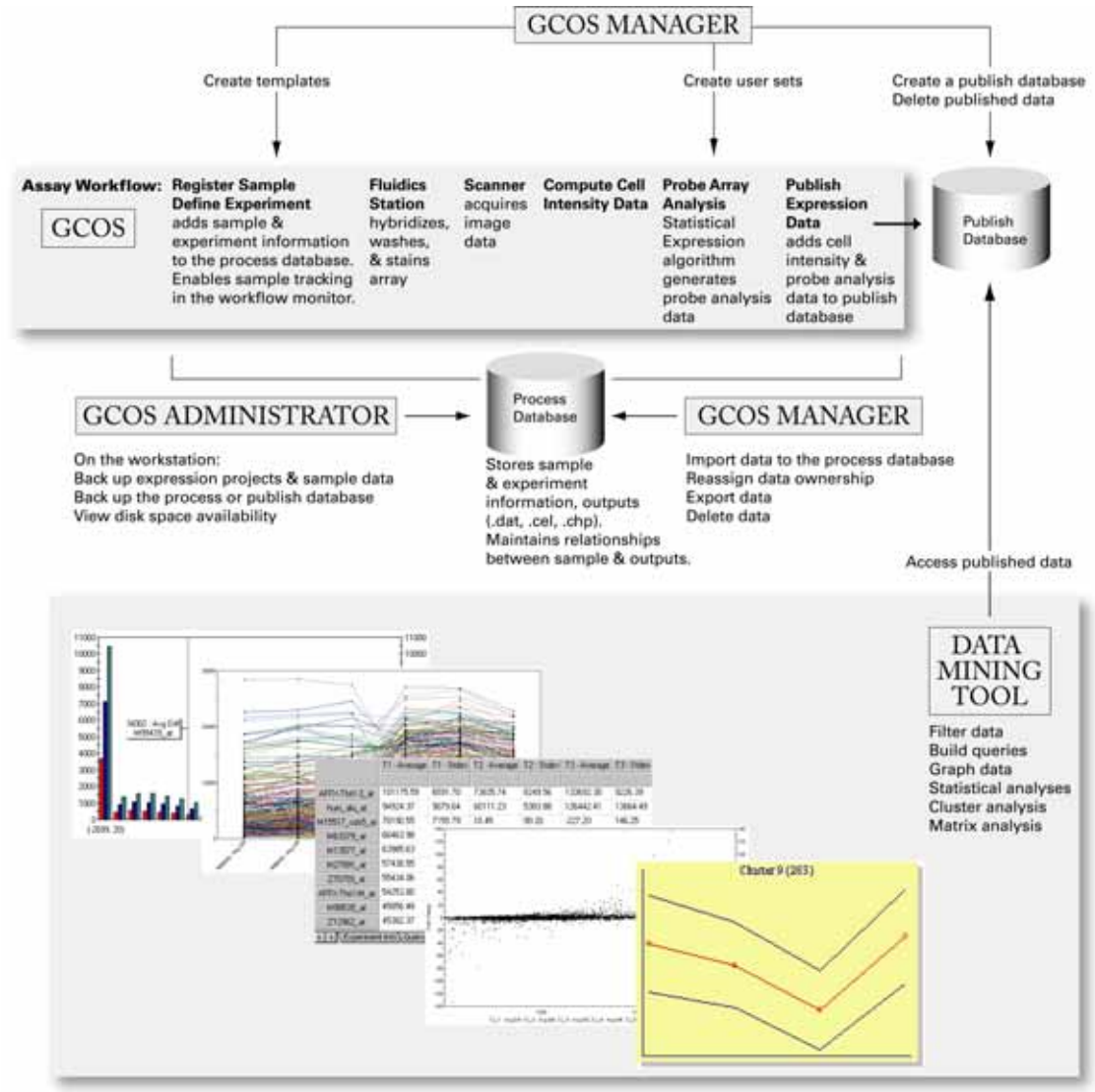
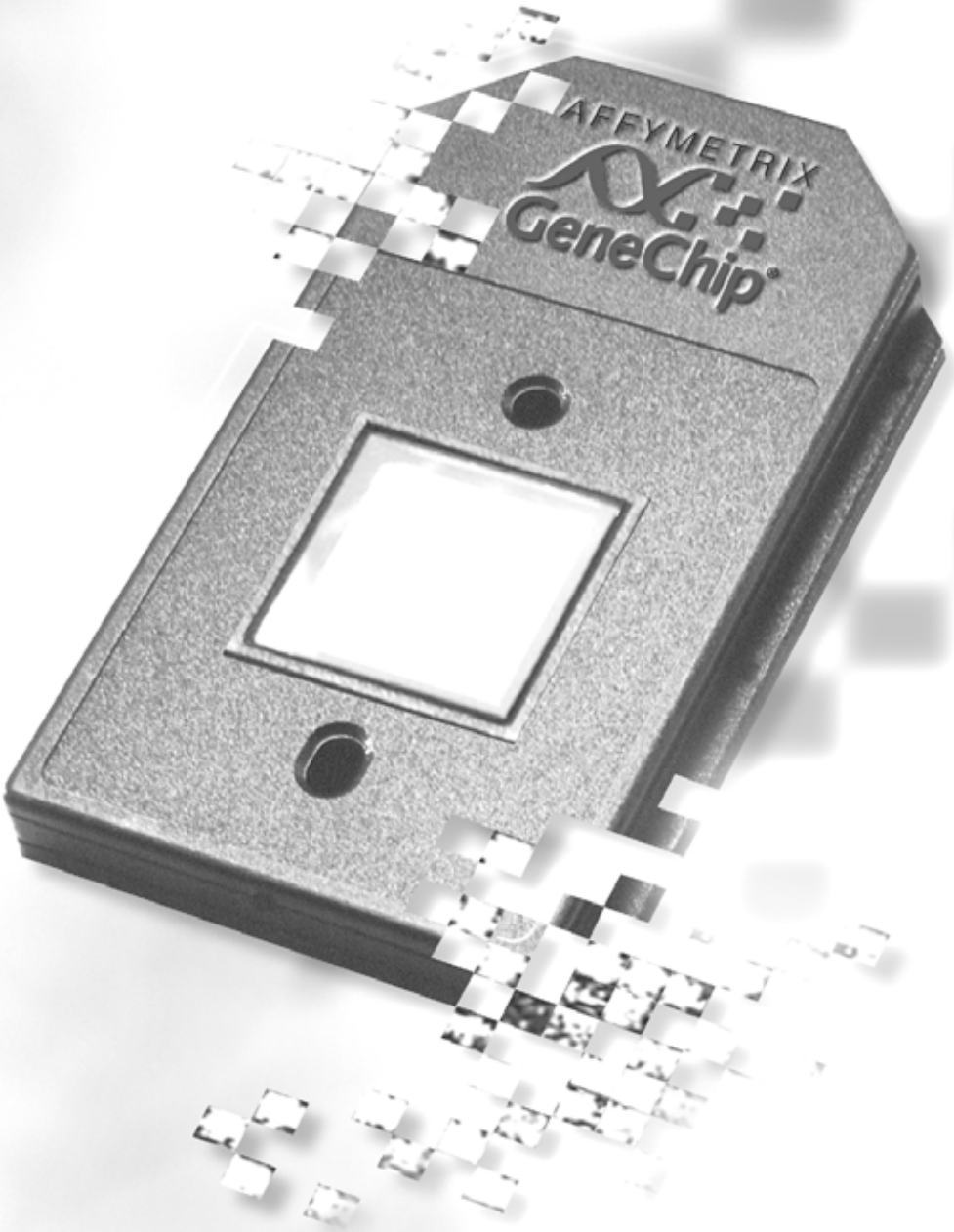


Figure 4.5
GCOS, GCOS Manager, GCOS Administrator, and Data Mining Tool software



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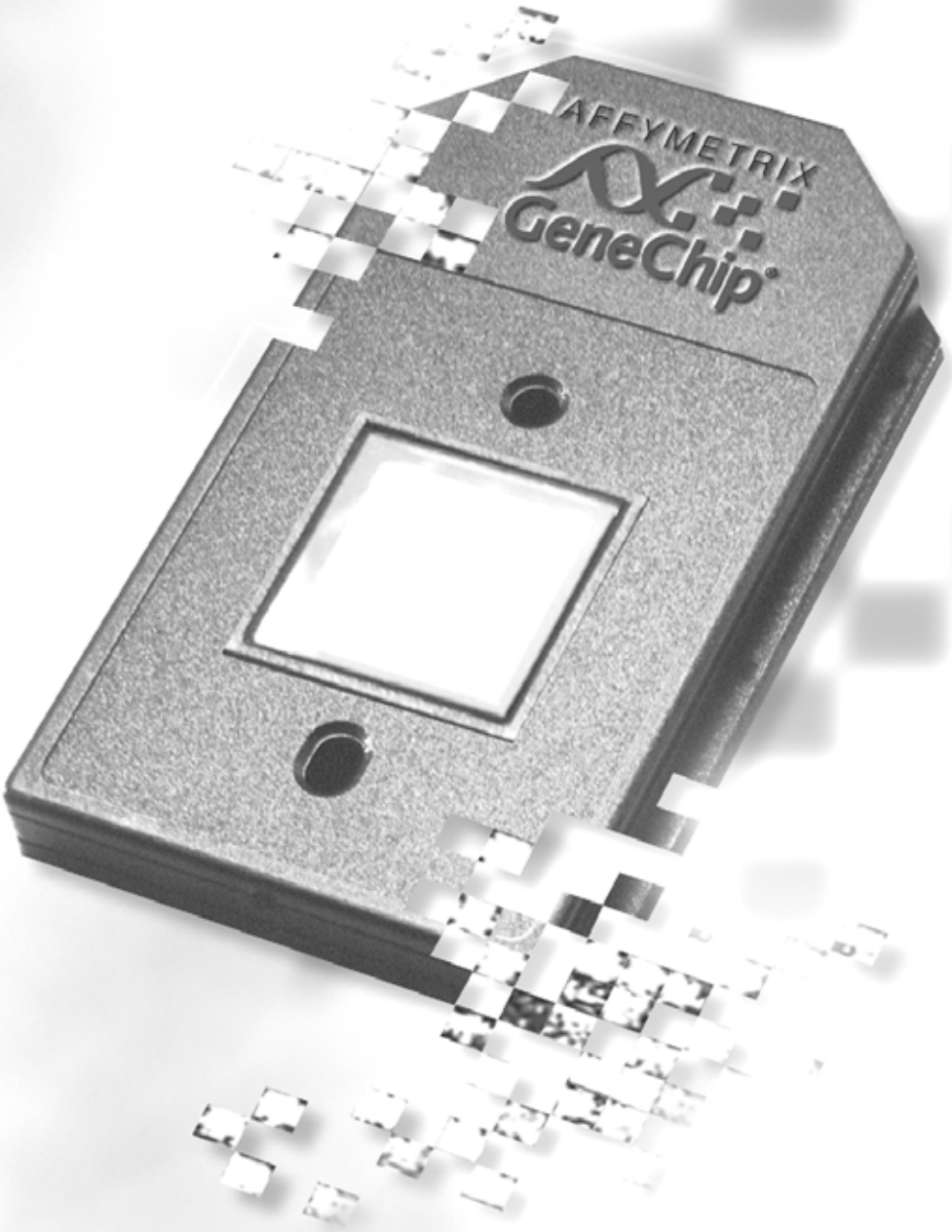
Chapter 5



Getting Started

This chapter explains how to:

- start the GeneChip® Operating Software (GCOS)
- configure the software to run with a remote Affymetrix® GeneChip Operating Software (GCOS) server
- specify filters that determine the data displayed by the system
- configure the fluidics station and scanner

Starting GCOS

1. Turn on the power for the computer workstation. If the workstation is connected to the instruments, also turn on the power for the GeneChip® Fluidics Station 400 and the Affymetrix® GeneChip® Scanner 3000 or the GeneArray® 2500 scanner.

WARNING

Laser in use during scanning.

Note

The scanner laser should be turned on and warmed up at least 10 minutes (if using the GeneChip® Scanner 3000) or 15 minutes (if using the GeneArray® 2500 scanner) before use. Refer to Appendix D, *Using the GeneChip® Scanner 3000*, on page 491 or to the *Agilent GeneArray Scanner User's Guide*.

2. After the computer startup is complete, press **Ctrl+Alt+Delete** to open the logon dialog box. Enter your user name, password, and domain.

3. Click the Windows **Start** menu button , then select **Programs** → **Affymetrix** →**GeneChip Operating Software**.

   When GCOS starts, the program displays the shortcut bar, data tree, and status log **(Figure 5.1)**.

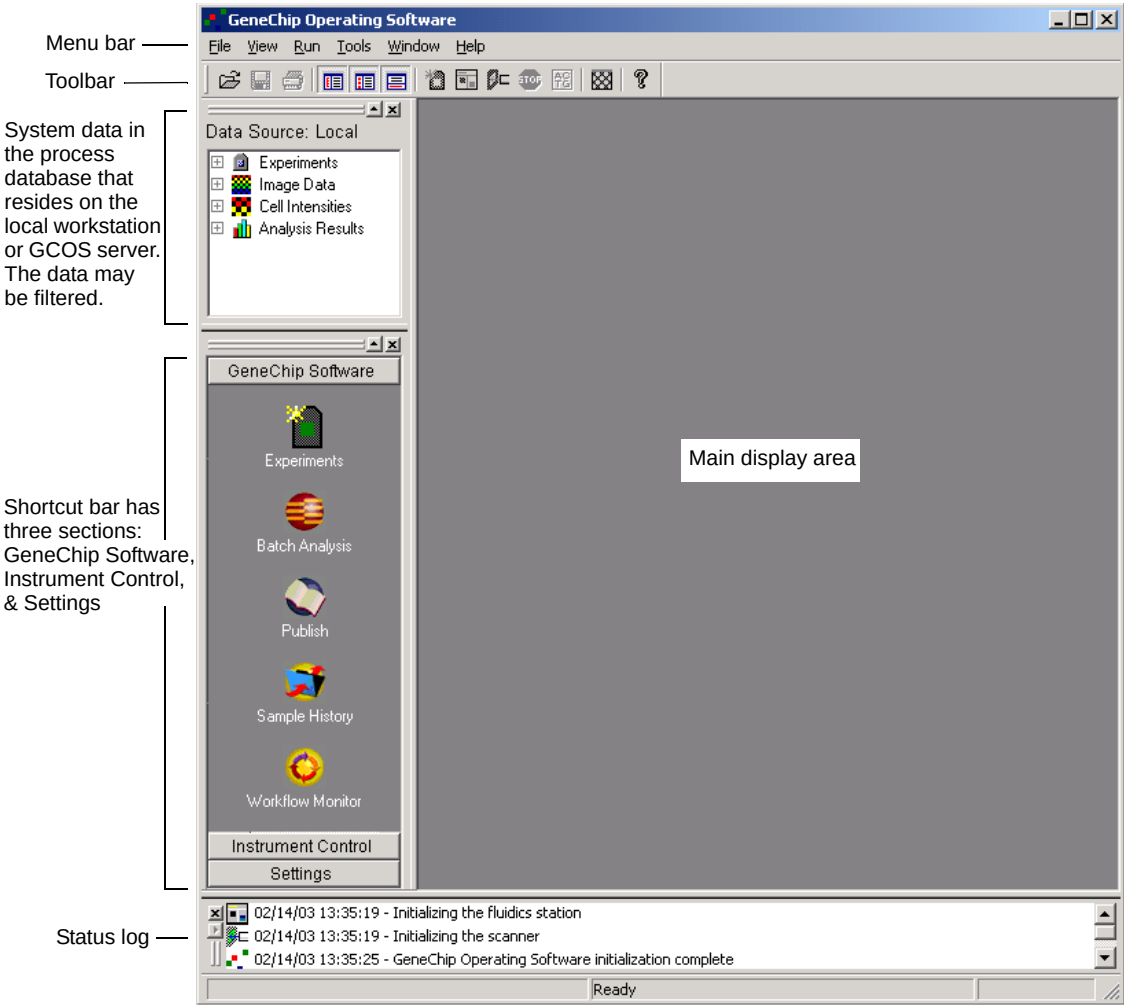


Figure 5.1
GCOS user interface (local database)

Configuring GCOS

The GCOS software may be configured to run with a network accessible GeneChip® Operating Software (GCOS) server.

Data Storage

Check the database setting to make sure GCOS is properly configured for your system (workstation or GCOS server database).

1. In the Settings shortcut bar, click the **Defaults** button . Alternatively, select **Tools →Defaults** from the menu bar.

   ⇒ The Defaults dialog box appears **(Figure 5.2)**.

2. Click the Database tab

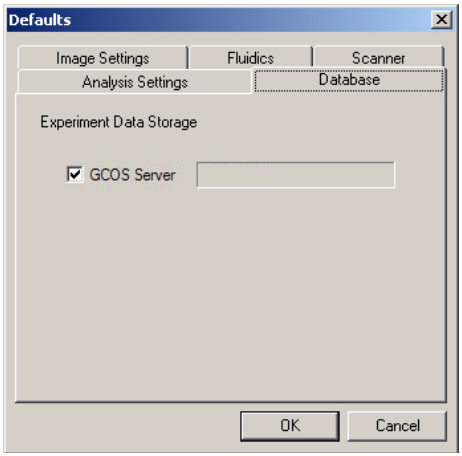


Figure 5.2
Defaults dialog box, Database tab

Note

The GCOS Server data storage option is available if all windows are closed and no instrument is active.

3. To store experiment data on the GCOS server, choose the **Affymetrix GCOS** option.

   If the **GCOS Server** option is not selected, the data are stored in the local MSDE database.

GCOS User Interface

Shortcut Bar

The shortcut bar provides quick alternatives to menu bar commands. The shortcut bar has three sections:

- GeneChip Software
- Instrument Control
- Settings

- To display a particular section of the shortcut bar, click the shortcut bar section name (**Figure 5.3** through **Figure 5.4**).

GeneChip Software Shortcut Bar

Figure 5.1 shows the GeneChip Software shortcut bar at startup. Additional buttons may be displayed in the shortcut bar, depending on the data types that are open **(Figure 5.3)**.

For example, if a scan image or cell intensity data are open, the shortcut bar includes the **Image Views** button . Click this button to view the cell intensity image in the main display area.

GeneChip® Software Shortcut Bar Functions

Click a button to change the display of open data in the main display area.

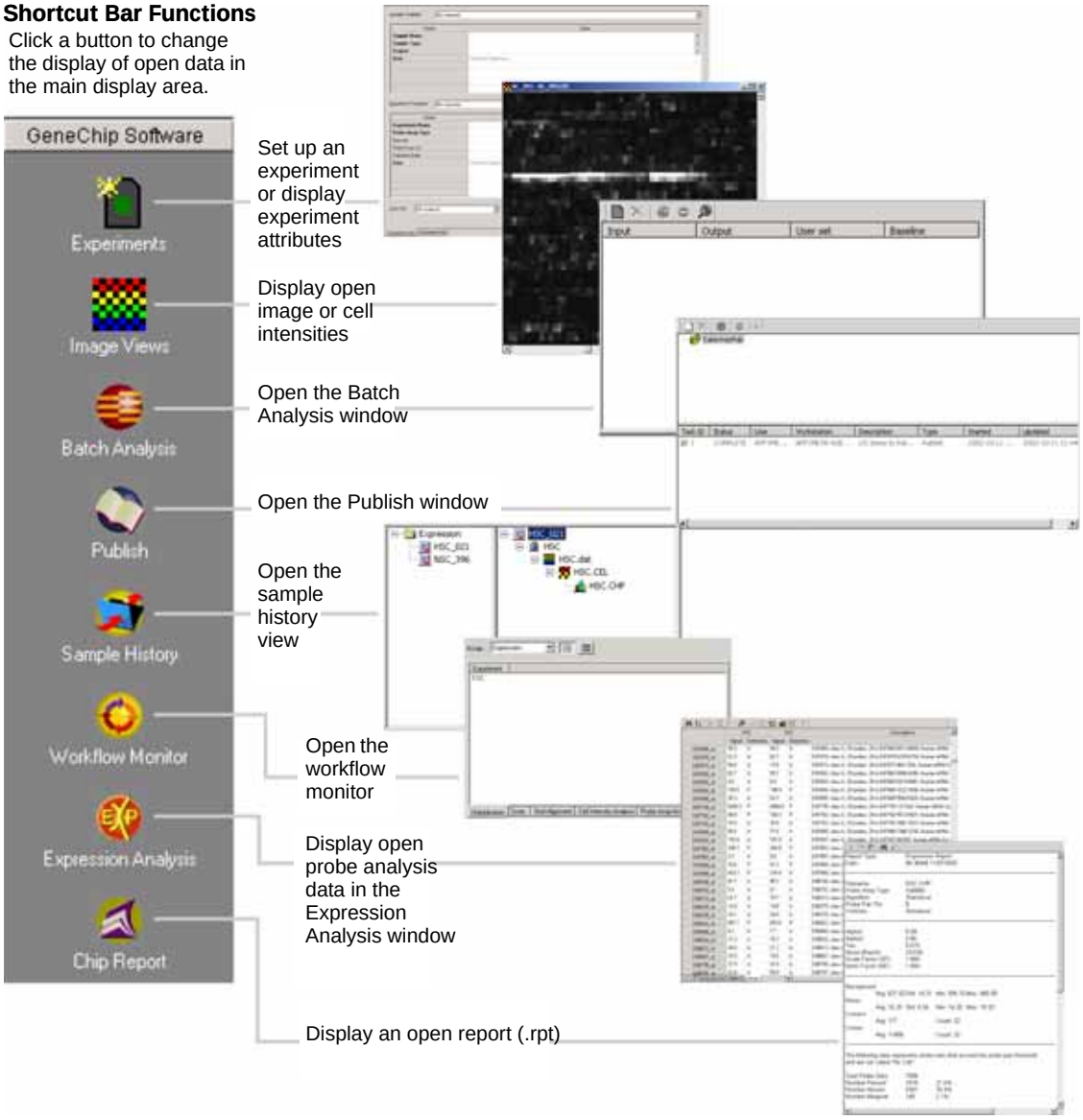


Figure 5.3
GeneChip Software shortcut bar

Instrument Control Shortcut Bar

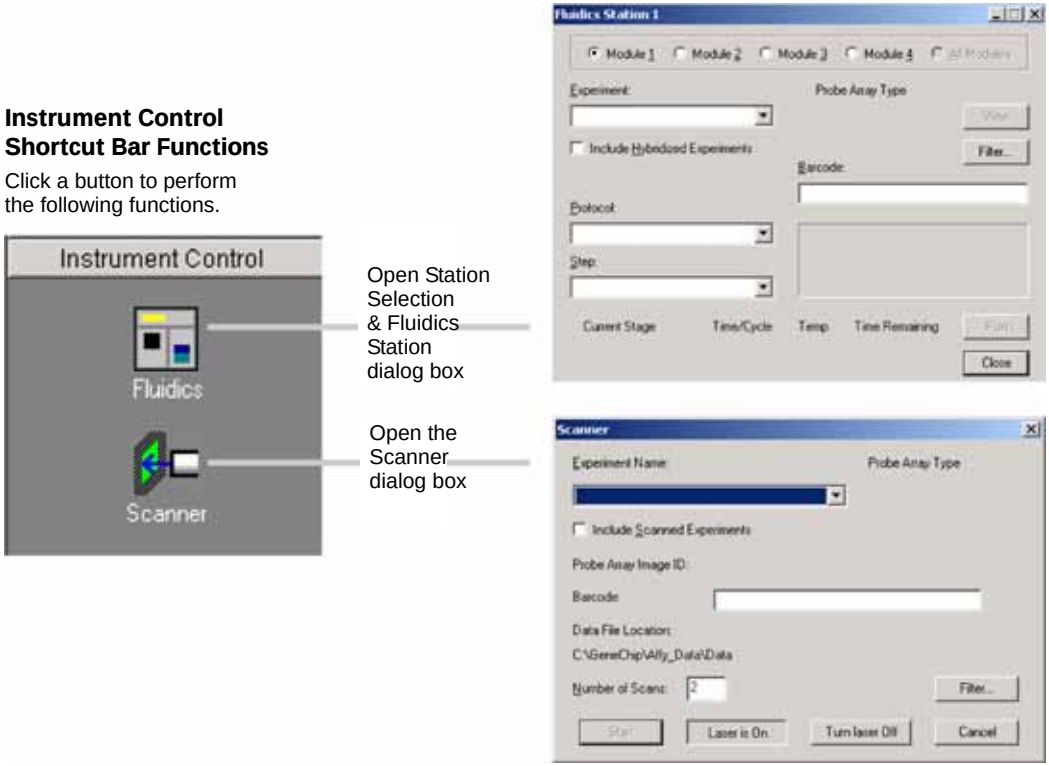


Figure 5.4
Instrument control shortcut bar

✔ Note

*The instrument control shortcut bar does not display instrument buttons if the workstation is not connected to the instruments, or if the default configuration settings specify no fluidics station or scanner installed (select **Tools** →**Defaults** from the menu bar to view the default settings).*

Settings Shortcut Bar

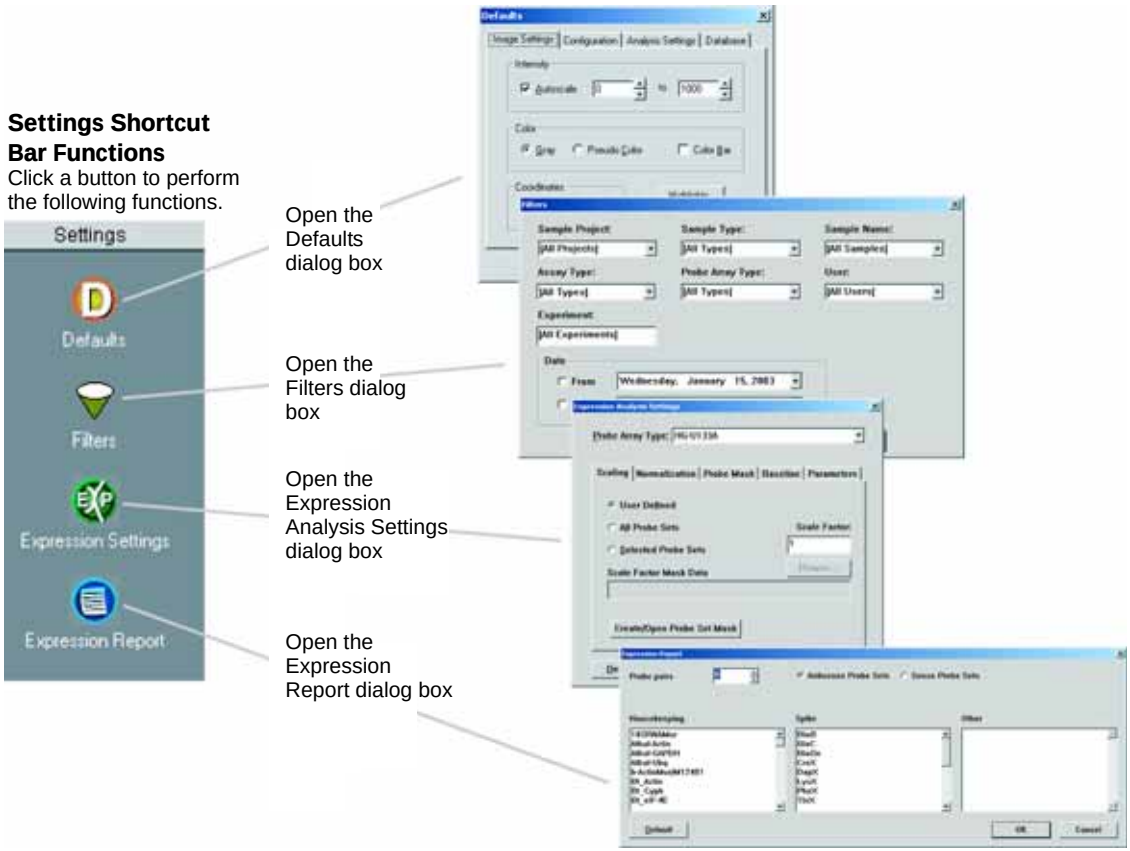


Figure 5.5
Settings shortcut bar

Data Tree

The data tree displays the data in the system (process database), including:

- experiments
- image data
- cell intensities
- analysis results

There are two different views of the data tree **(Figure 5.6)**:

- Classic View organizes the experiment by data type.
- Project/Sample view organizes the experiment data by sample, and samples are organized by project.

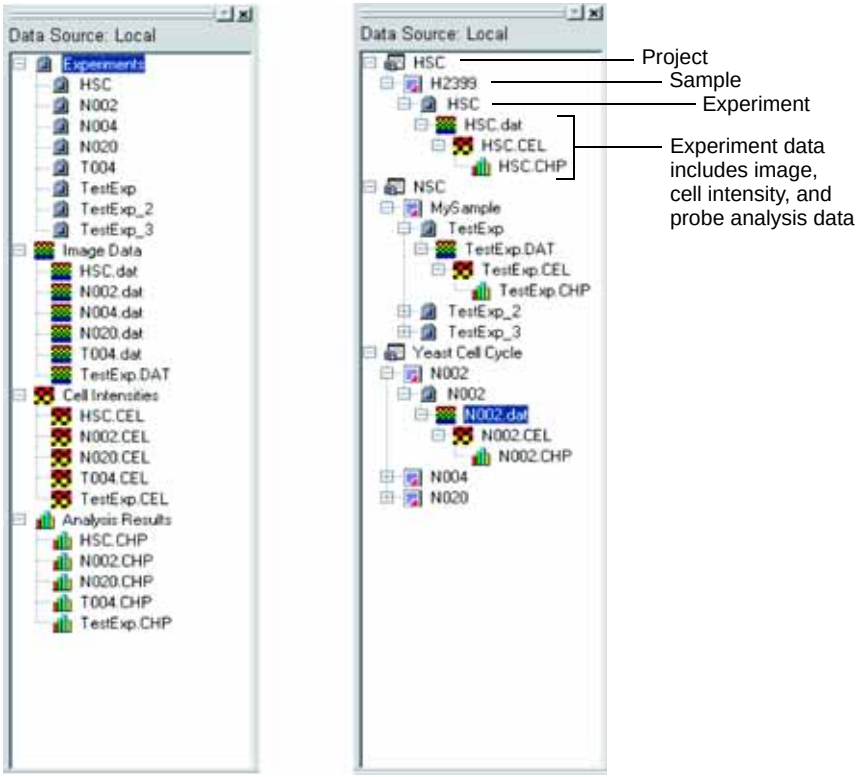


Figure 5.6
Data tree, Classic View (left), Project/Sample View (right)

Data Tree Display Options

- To select the data tree view, right-click the data tree and choose **Classic View** or **Project/Sample View** from the shortcut menu that appears.
- To collapse or expand the list of data types in the tree, click the minus [-] or plus [+] sign next to each data type icon.
- To hide (or display) the data tree, click the **Data Tree** button .

Data Tree Shortcut Menu

- To view a shortcut menu of commands (specific to the data type), right-click a data name in the tree **(Figure 5.7)**.

Figure 5.7
Data tree shortcut menu for cell intensities

From the data tree you can:

- open an experiment, an image, cell intensity data, or probe analysis data
- update the data source tree to show new information in the system
- display information about the data (attributes)
- rename an experiment, sample, or project
- analyze an image (generate the cell intensity data)
- analyze the cell intensity data from an expression probe array (generates the probe analysis data)
- generate an expression report

Status Log

The status log displays system status messages **(Figure 5.1)**.

1. To clear the messages, right-click the status log and select **Clear Messages** in the shortcut menu that appears.
2. To mute the error message sound, right-click the status log and remove the check mark from the **Play Error Sound** option.
3. Click the **Status Log** button in the main toolbar to hide (or display) the status log.

Renaming a Project, Sample, or Experiment

In the data tree project/sample view, you can rename an project, sample, or experiment. In the data tree classic view, you can rename an experiment.

1. Right-click the data tree, and select **Project/Sample View** from the shortcut menu that appears **(Figure 5.8)**.

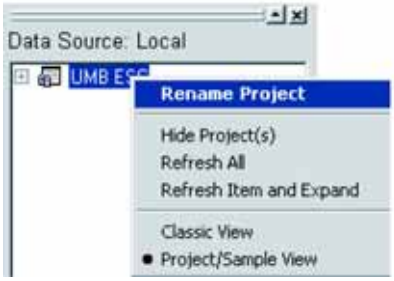


Figure 5.8
Data tree, project/sample view

2. Expand the data tree, and right-click the item (project, experiment, or sample) that you want to rename.
3. Select **Rename Project** (**Rename Experiment** or **Rename Sample**) in the shortcut menu that appears.

   ⇒ A Rename dialog box appears **(Figure 5.9)**.

Figure 5.9
Rename Project dialog box

4. Enter a new name and click **OK**.

GCOS Data

There are three types of GCOS data.

- Probe information or *library* files are unique for each probe array type and contain information about the probe array design characteristics, scanning parameters, and default analysis parameters.
- Fluidics protocol files define the hybridization, wash, or stain protocols run by the GeneChip® Fluidics Station.
- Experiment data include the sample and experiment created by the user when an experiment is defined, as well as the other data types GCOS generates during an analysis: image data, cell intensity data, and probe analysis data.

For more information about the data types, see Appendix F, *GCOS Data Types*, on page 567.

Opening Data and Viewing Information

You can view information (attributes) about experiments, images, cell intensity data, and probe analysis data.

1. In the data tree, double-click the data name. Alternatively, right-click the data name in the tree and select **Open** from the shortcut menu that appears.

   ⇒ This displays the data in the main display area.

Many data types may be open at the same time, but you can view only one at a time in the main display area (except for a cascade or tile view of images or cell intensity data).

2. Click a GeneChip Software shortcut bar button **(Figure 5.3)** to toggle the view between the different types of open data.

3. To view information about the data, right-click the data name in the data tree and select **Information** in the shortcut menu that appears.

   ⇒ An Information dialog box appears (**Figure 5.10** and **Figure 5.11**).

4. To copy experiment data information to the system clipboard, click **Copy**.

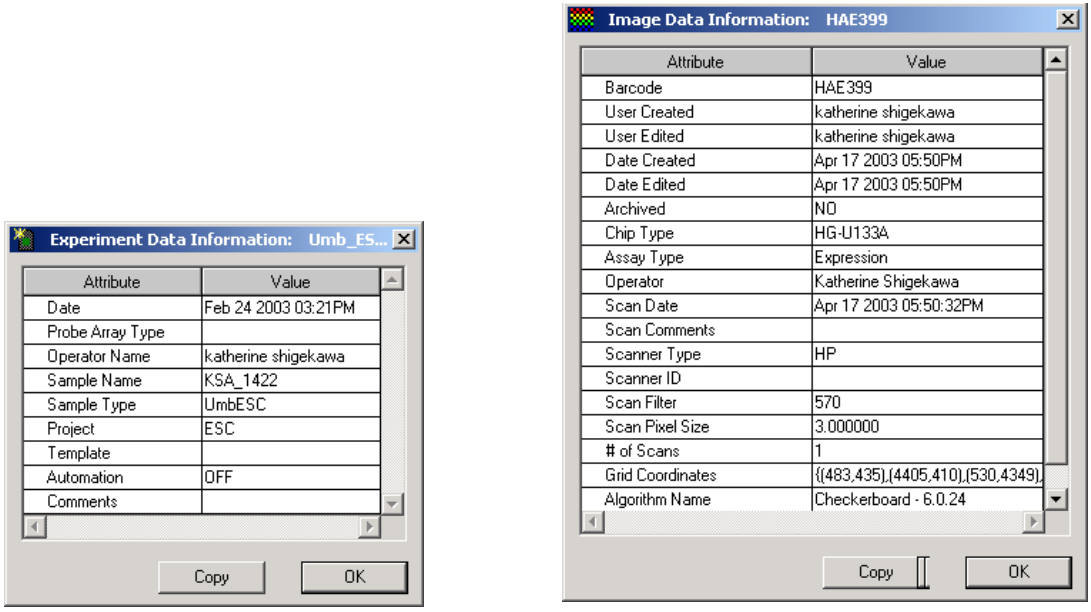
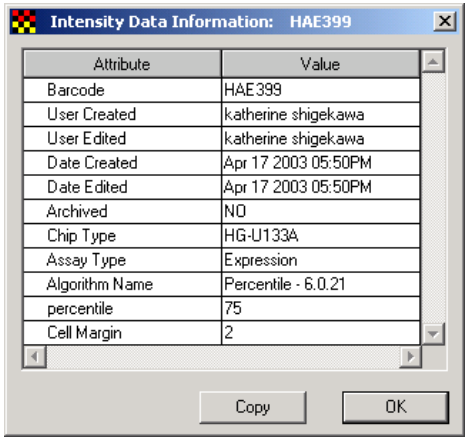


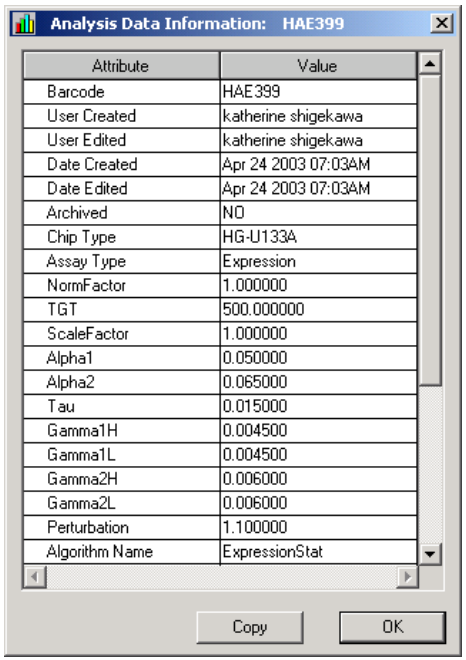
Figure 5.10
Experiment information (left) and image information (right)



Intensity Data Information: HAE399

Attribute	Value
Barcode	HAE399
User Created	katherine shigekawa
User Edited	katherine shigekawa
Date Created	Apr 17 2003 05:50PM
Date Edited	Apr 17 2003 05:50PM
Archived	NO
Chip Type	HG-U133A
Assay Type	Expression
Algorithm Name	Percentile - 6.0.21
percentile	75
Cell Margin	2

Copy OK



Analysis Data Information: HAE399

Attribute	Value
Barcode	HAE399
User Created	katherine shigekawa
User Edited	katherine shigekawa
Date Created	Apr 24 2003 07:03AM
Date Edited	Apr 24 2003 07:03AM
Archived	NO
Chip Type	HG-U133A
Assay Type	Expression
NormFactor	1.000000
TGT	500.000000
ScaleFactor	1.000000
Alpha1	0.050000
Alpha2	0.065000
Tau	0.015000
Gamma1H	0.004500
Gamma1L	0.004500
Gamma2H	0.006000
Gamma2L	0.006000
Perturbation	1.100000
Algorithm Name	ExpressionStat

Copy OK

Figure 5.11
Cell intensity data information (left) and probe analysis data information (right)

Filtering Data

You may apply filters to the experiment data. The filters determine the data that GCOS displays in the data tree, the sample history view, workflow monitor, and the instrument control dialog boxes.

Filters are applied on a per user basis (identified by the logon name). The filters you specify do not affect the filters specified by other users.

1. Select **Tools →Filters** from the menu bar.

   ⇒ The Filters dialog box appears **(Figure 5.12)**.

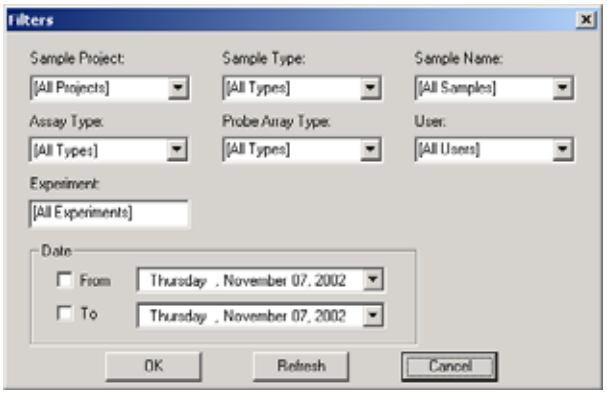


Figure 5.12
Filters dialog box

2. Make selections from the drop-down lists to specify the filters.
3. If you want to view the experimental data for a specific time period, choose the **Date From** or **To** options and click the drop-down arrows to select calendar dates.
4. Click **OK** when finished to close the Filters dialog box and apply the filters.

   ⇒ The status bar indicates that filters are applied **(Figure 5.13)**.

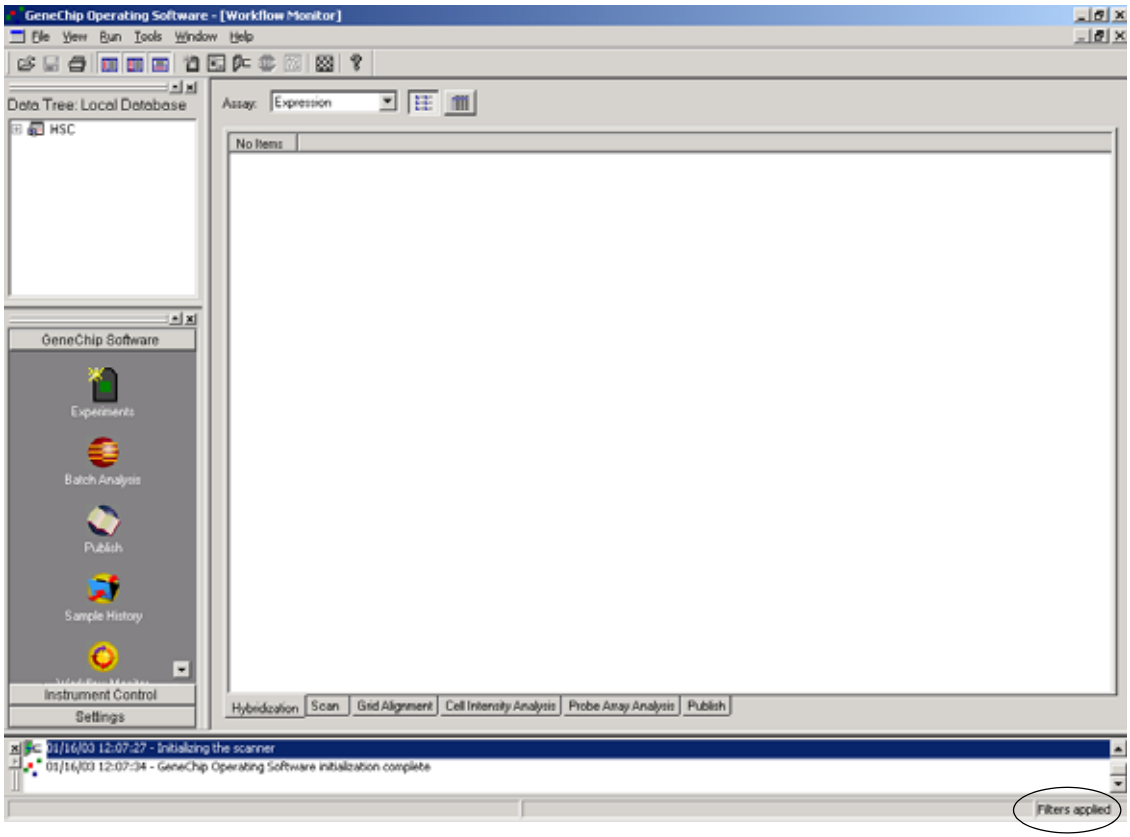


Figure 5.13
Status bar in the lower right indicates filters applied

Configuring the Fluidics Station & Scanner

Before you use the fluidics station, check the fluidics station configuration and prime the fluidics station with appropriate buffer. For information on how to prime the fluidics station, and set up and run a fluidics protocol, see Chapter 8, *Controlling the Instruments*, on page 97.

Configuring the Fluidics Station

Before running a fluidics protocol, check to make sure the fluidics station(s) is properly configured.

1. Select **Tools →Defaults** from the menu bar.

   ⇒ The Defaults dialog box appears **(Figure 5.14)**.

Figure 5.14
Defaults dialog box, Fluidics tab

2. Click the Fluidics tab.
3. Confirm the number of fluidics stations installed is correct or enter a new value.
4. If desired, check mark **Notification Message when protocol is complete** to display a notification message when a fluidics protocol is completed.

5. Click **OK** to close the Defaults dialog box.

Configuring the Scanner

1. Select **Tools →Defaults** from the menu bar.
   ⇒ The Defaults dialog box appears **(Figure 5.15)**.

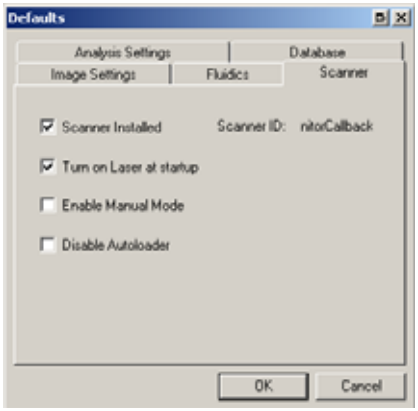
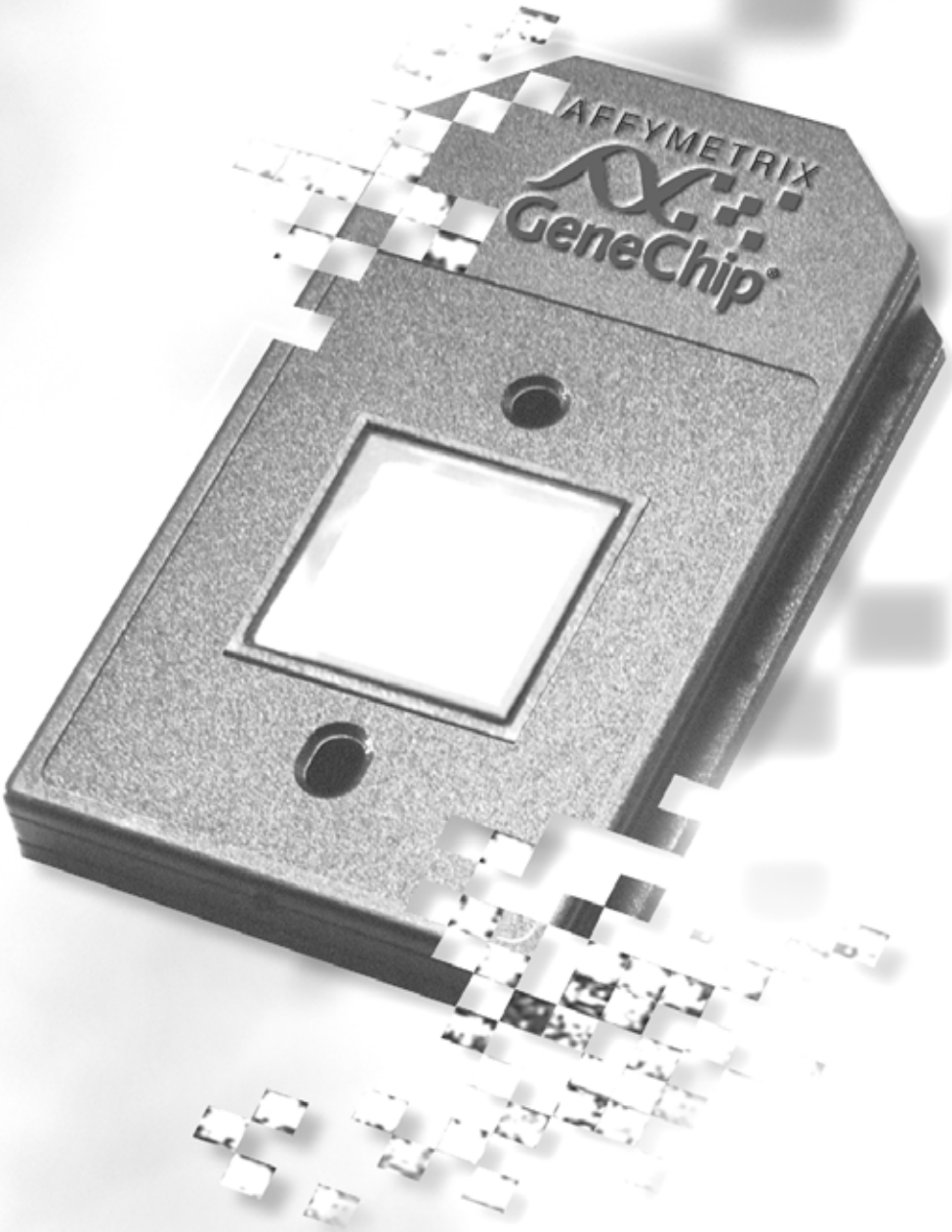


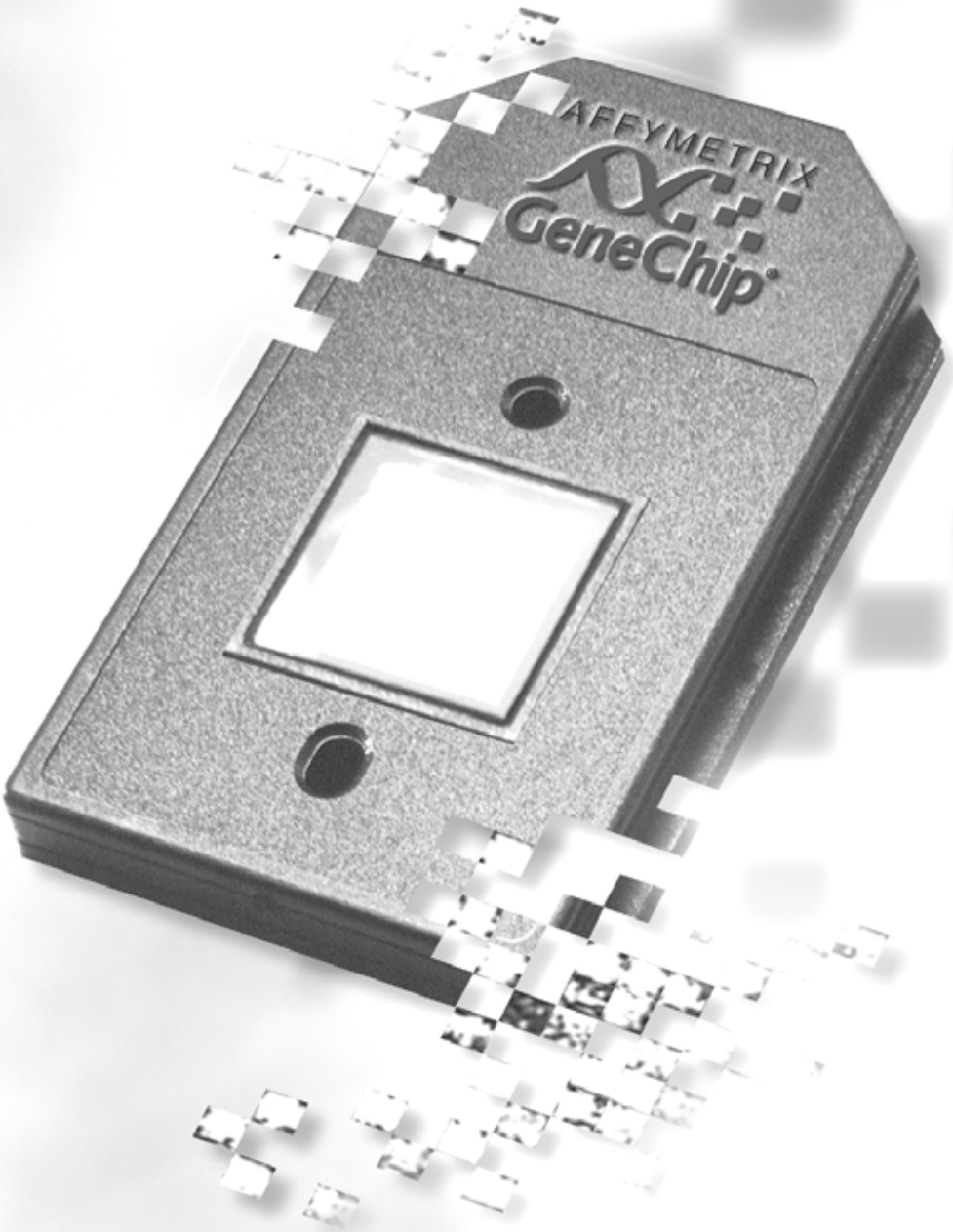
Figure 5.15
Defaults dialog box, Scanner tab

2. Click the Scanner tab.
3. If the workstation is connected to the scanner, choose the **Scanner Installed** option.
4. To automatically turn the scanner laser on at startup, choose the **Turn on Laser at startup** option.
5. If the AutoLoader is present and you would like to disable the autorun feature and add cartridges one by one, choose the **Enable Manual Mode** option.
6. If the AutoLoader is present and you would like to disable it and use the GCOS software without the AutoLoader, choose **Disable AutoLoader** option.



6

Chapter 6



Setting Up an Experiment

This chapter explains how to set up an experiment. You must define an experiment in GCOS before processing a probe array in the fluidics station or scanning a probe array. GCOS relies on the experiment information to direct the scanner and apply the correct cell and data analysis algorithms.

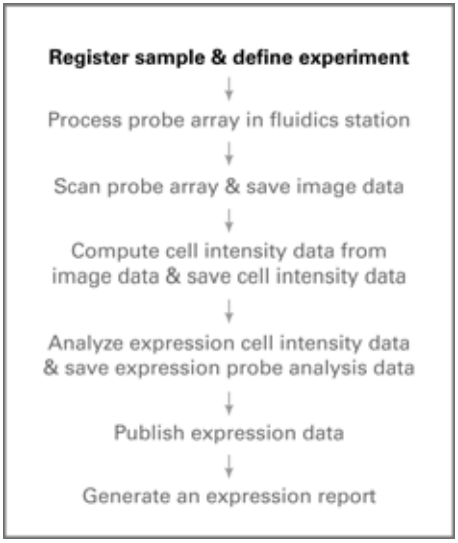


Figure 6.1
Assay & analysis flow chart

Registering a Sample & Defining an Experiment

You identify, or register, a sample by entering sample information (*attributes*) and associating the sample with a project (which may include several different samples) **(Figure 6.3)**.

To define an experiment, specify the type of probe array to which the target (sample) will be hybridized as well as other information relevant to the experiment. You may define one or more experiments per sample (for example, the different probe arrays of a multiple probe array set hybridized to the same sample).

You can associate a barcode with an experiment. If you entered an Affymetrix® barcode (manually or using a keyboard wedge reader) GCOS automatically reads and displays the probe array type, lot number, and the expiration date. GCOS uses the experiment information to select scanner specifications for the probe array and identify the user-modifiable algorithm parameters for data analysis.

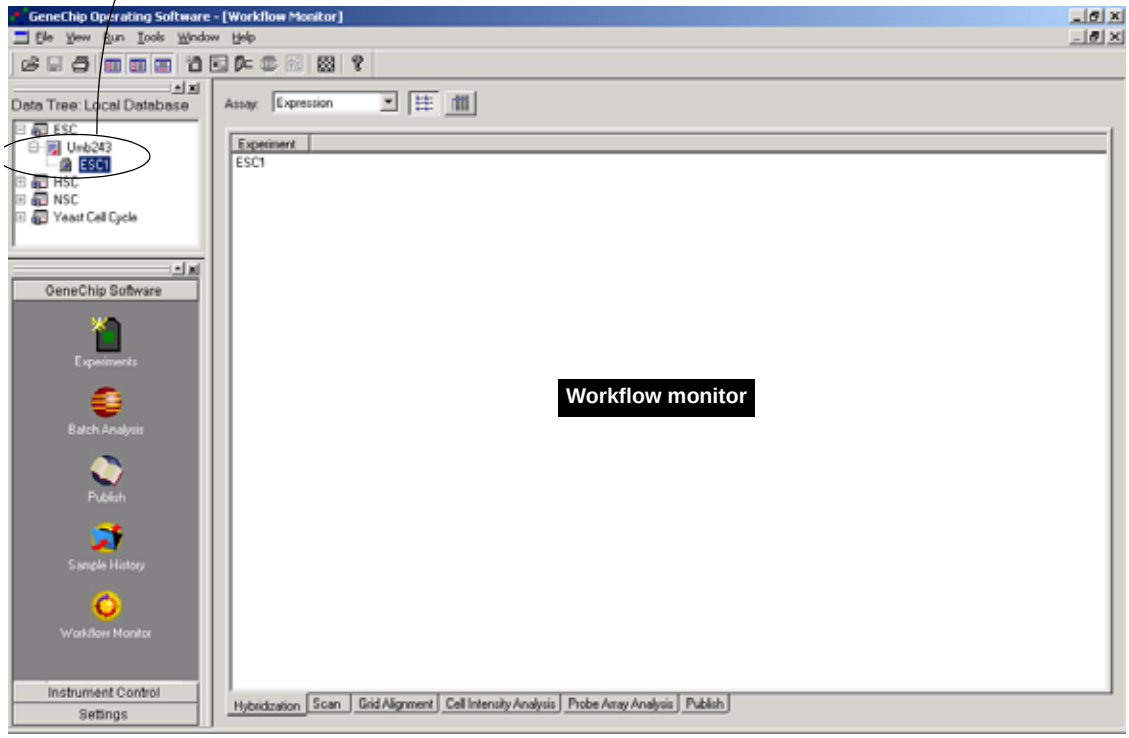
You can also use a template (defined in GCOS Manager) to register a sample and define an experiment. For more information on creating a template, see *Template Tab*, on page 344.

GCOS automatically adds the sample and experiment information to the process database. This enters the experiment in the workflow and enables you to track its status in the workflow monitor. After you define an experiment, the Hybridization tab of the workflow monitor displays the experiment and associated sample **(Figure 6.2)**. The Hybridization tab displays experiments that have not yet been processed in the fluidics station. See *Workflow Monitor*, on page 90 for more information about the workflow monitor.

The software uses sample and experiment information to maintain relationships among the experiment data (image, cell intensity data, probe analysis data), manage the workflow queue, and identify the user-modifiable algorithm parameters for data analysis.

✔ Note *GeneChip® Operating Software Server supports expression, Resequencing, Mapping, and Universal data.*

Sample Umb 243 is registered and associated with the ESC project.
Experiment ESC1 is defined and entered into the workflow.



Workflow monitor tabs. Hybridization tab shows the ESC1 experiment is defined, but not yet processed in the fluidics station.

Figure 6.2
Workflow monitor, hybridization tab

1. In the GeneChip Software shortcut bar, click **Experiment Info** , or select **File →New Experiment** from the menu bar.

   ⇒ The Experiment Information tab appears **(Figure 6.3).**

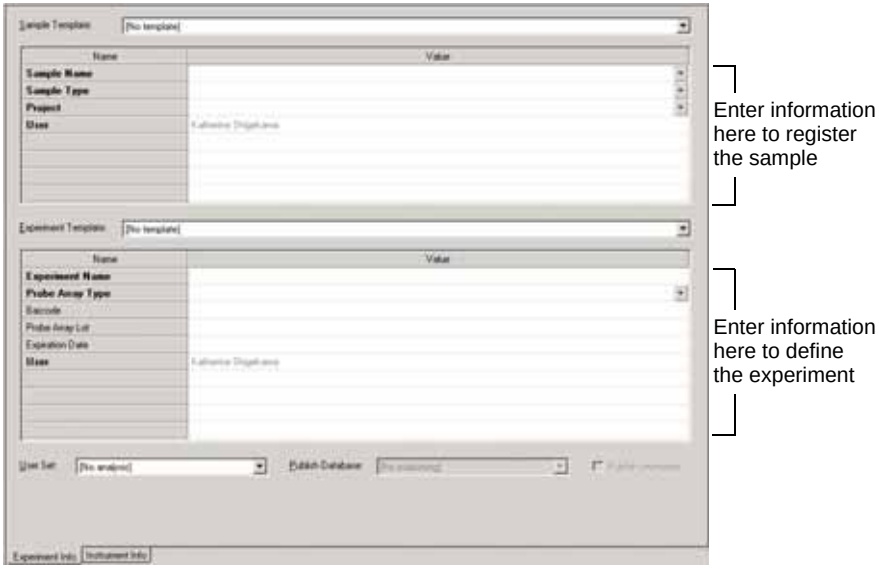


Figure 6.3
Experiment Information tab

Note *In the Experiment Information tab, items in bold are required entries.*

2. Enter a new sample name (up to 64 alphanumeric characters) or select an existing sample name from the drop-down list. This is required by GCOS. If you enter a new sample name, the sample type and project are also required.

   Or, if you want to use a sample template, make a selection from the **Sample Template** drop-down list and enter the sample information. A template specifies the information fields available in the Experiment Information tab. You can define a template in GCOS Manager. For more information on creating templates, see *Template Tab*, on page 344.

3. Enter a unique experiment name (up to 64 alphanumeric characters). This is required by GCOS. Or, if you want to use an experiment template, make a selection from the **Experiment Template** drop-down list and enter the experiment information.

The experiment name also serves as the name for the subsequent data types generated during the analysis. See Appendix F, *GCOS Data Types*, on page 567 for a description of GCOS data types.

4. Make a selection from the **Probe Array Type** drop-down list (required by GCOS).

5. You may add a barcode (up to 64 characters). Enter a barcode value in the dialog box, or use a barcode reader to read the barcode directly from the probe array cartridge.

   If you use a barcode reader to read an Affymetrix® barcode from the probe array cartridge:

   - GCOS automatically fills in the probe array type, probe array lot and expiration date fields
   - the Probe Array Type drop-down list is disabled

✔ Note

*GCOS has the ability to store barcodes. You can enter barcode information in either of two places: in the Experiment tab (**Figure 6.3**) or in the Fluidics dialog box (see **Figure 8.4** on page 101). When you enter a valid barcode (maximum of 64 characters), GCOS stores the barcode with the experiment.*

6. To automatically publish expression probe analysis data:

   a. Make a selection from the **User Set** drop-down list.

   A user set is defined in GCOS Manager. The userset specifies the values for the user-modifiable expression algorithm parameters. For more information about user sets, see *Userset Tab*, on page 335.

   b. Make a selection from the **Publish Database** drop-down list. See Publishing Data on page 245 for more information about publishing data.

✔ Note

*You must specify both a user set and a publish database, otherwise the probe analysis data will not be published. If you specify only a user set, the analysis proceeds using the analysis settings in the Expression Analysis Settings dialog box, but the data are not published. To view the Expression Analysis Settings dialog box, click **Expression Settings** in the Settings shortcut bar.*

   c. To include the cell intensity data in the publish database, choose the **Publish Intensities** option.

✔ Note

Publishing cell intensity data uses large amounts of computer memory. For example, publishing the probe analysis data for a high density probe array requires approximately 2 MB of disk space compared to 30 MB for both the probe analysis and cell intensity data.

7. Click the **Save** button .

   ⇒ This saves the experiment and displays it in the data tree.

✔ Note

If you specified a user set and publish database during experiment setup, the software prompts you for the publish database password before the experiment is saved.

After the probe array is processed in the fluidics station and scanned, click the Instrument Info tab to view information about the fluidics protocol and scanning parameters captured by GCOS **(Figure 6.4)**.

The instrument information page is blank until the probe array has been processed in the fluidics station.

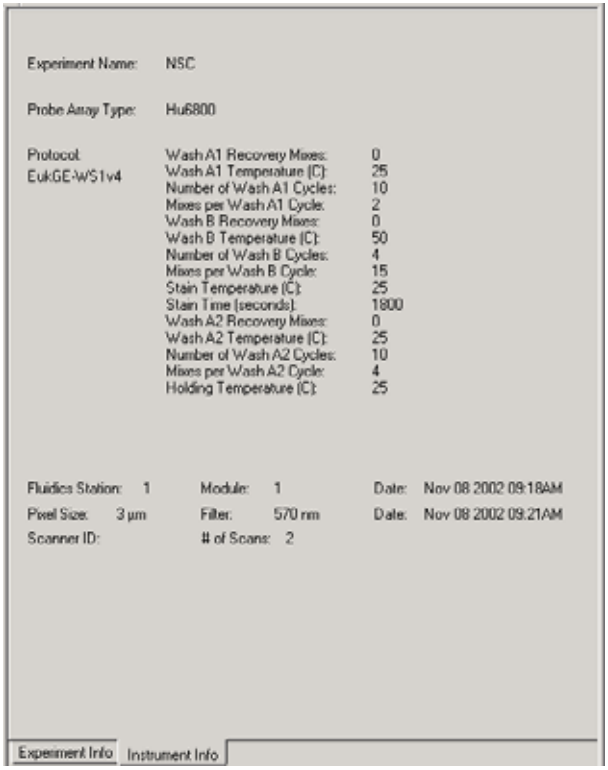


Figure 6.4
Instrument Info tab

Viewing or Editing an Experiment

1. To display an experiment, double-click the experiment name in the data tree. Alternatively, right-click the experiment name and select **Open** from the shortcut menu that appears.
2. To edit an entry, highlight the entry and enter the new information. Alternatively, right-click to open a shortcut menu of edit commands **(Figure 6.5)**.

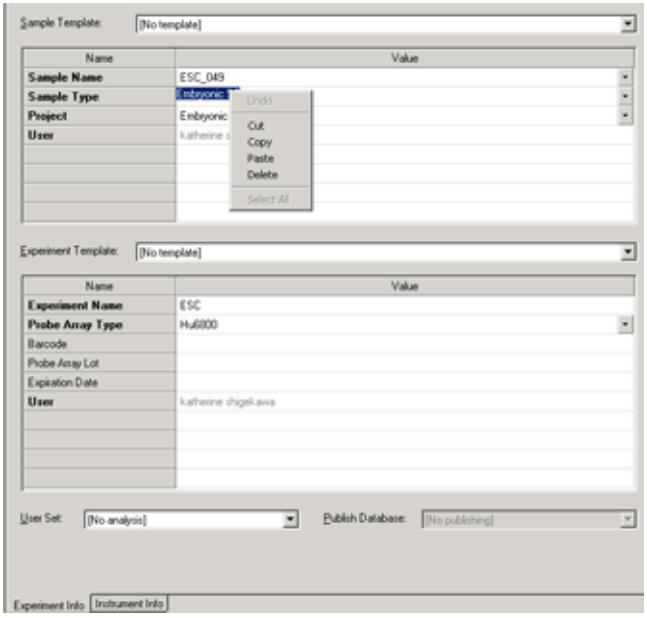


Figure 6.5
Experiment information and shortcut menu of edit commands

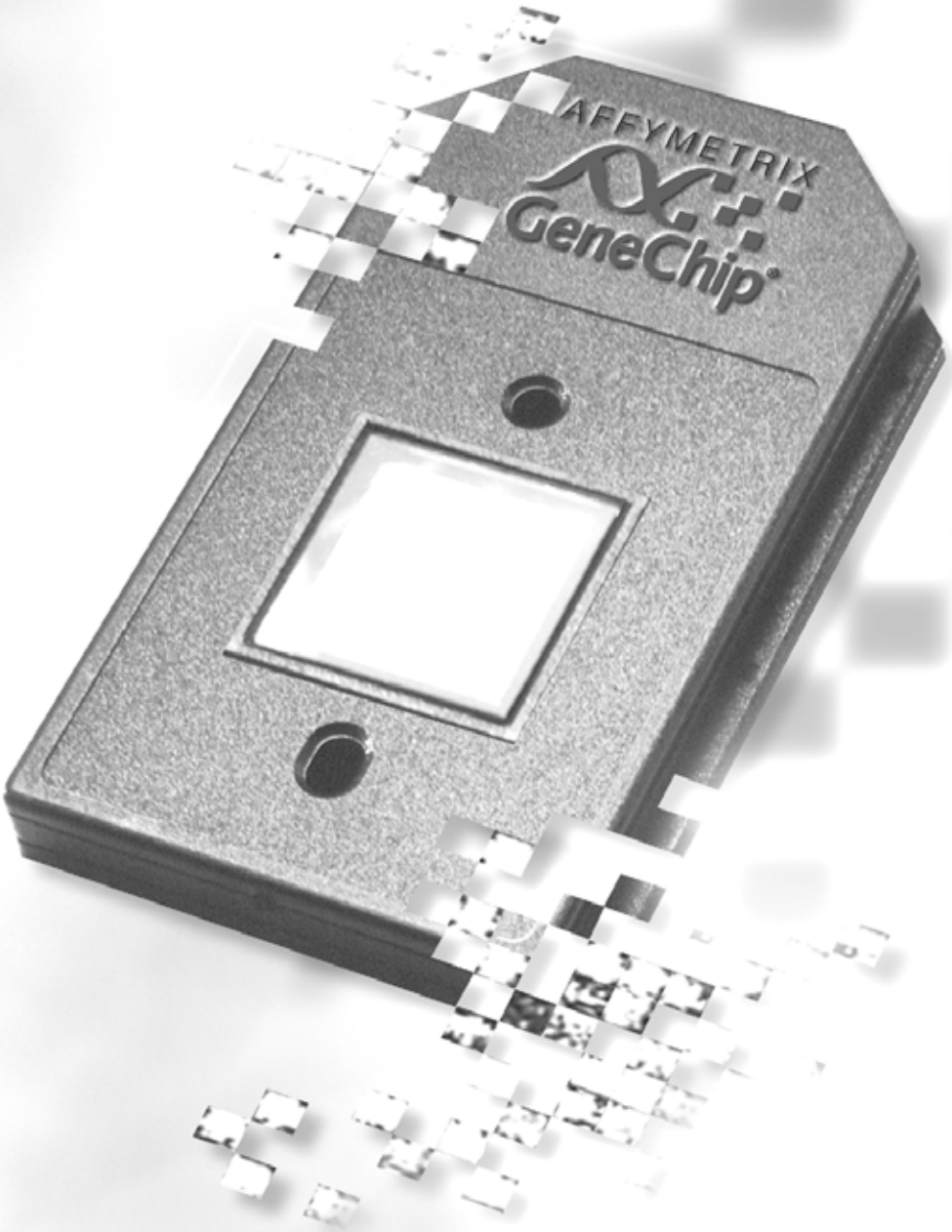
3. To clear all fields, select **Edit →Clear** from the menu bar (equivalent to closing the experiment without saving).

✔ Note

When connected to a GCOS server database, only users authorized by the System Administrator may delete data from the system using the GCOS Manager software. When operating using the local MSDE database, there is no data security.

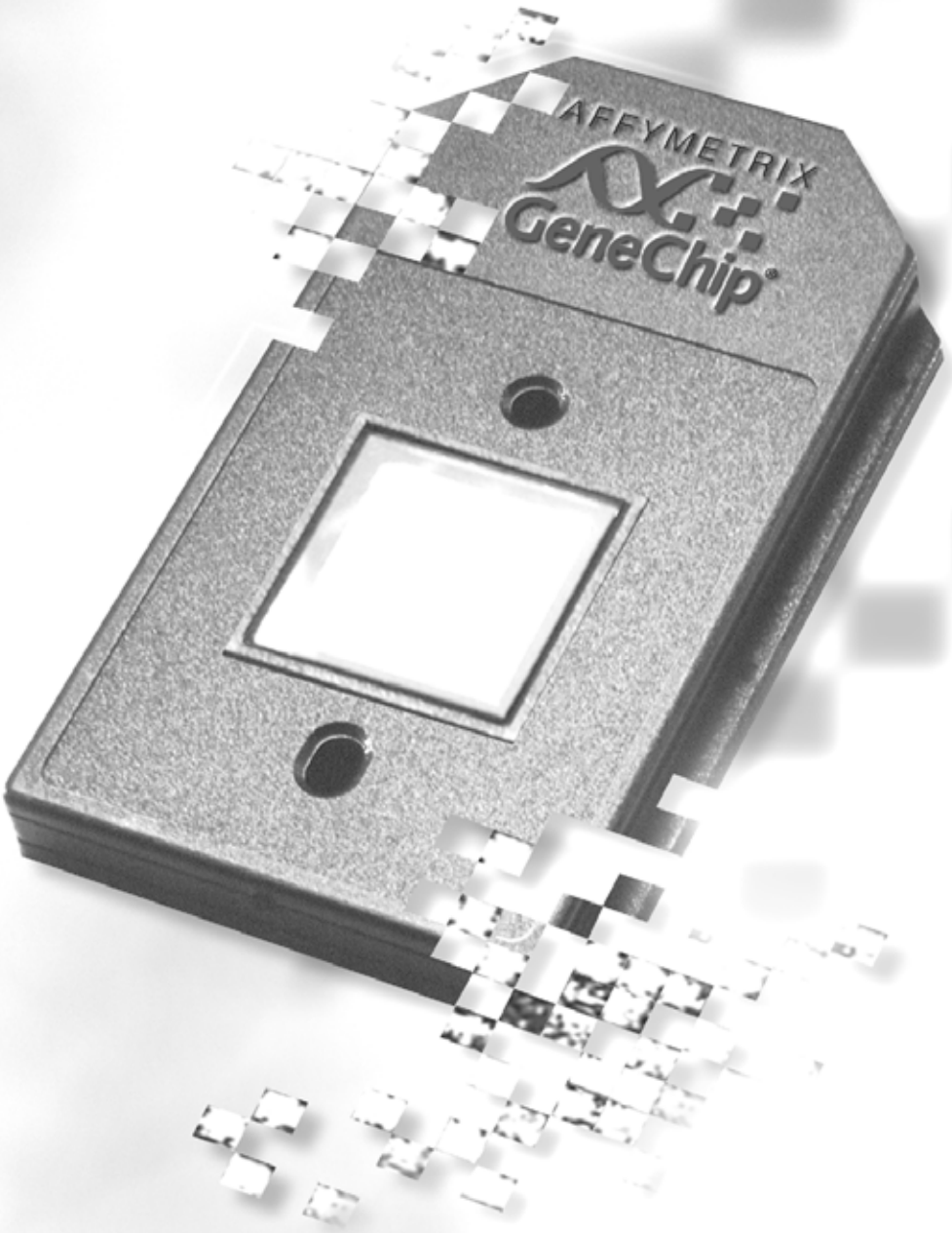
4. When finished, click the **Close** button X in the upper right corner of the window or select **File →Close** from the menu bar.

   Before the experiment is closed, GCOS prompts you to save changes.



7

Chapter 7



Sample History & Workflow Monitor

This chapter explains how to use the sample history feature and workflow monitor to track samples and experiment data.

Sample History

The sample history feature provides two different views of the samples in the system:

- **Data view** shows the experiment data derived from a particular sample
- **Process view** shows all stages (sample registration, experiment setup, hybridization, scan, grid alignment, cell intensity analysis, probe array analysis, and publishing) that are completed or pending for a sample

Displaying Sample History

1. In the GeneChip Software shortcut bar, click **Sample History**.

   ⇒ The sample history data view appears **(Figure 7.1)**.

   The data view is the default. If the data view is not displayed, select **View →Data** from the menu bar.

   The sample tree displays all assay samples. The expression folder contains all registered expression analysis samples.

✔ Note

If filters have been applied, not all registered samples may be displayed. See Filtering Data on page 65 for more information about filters.

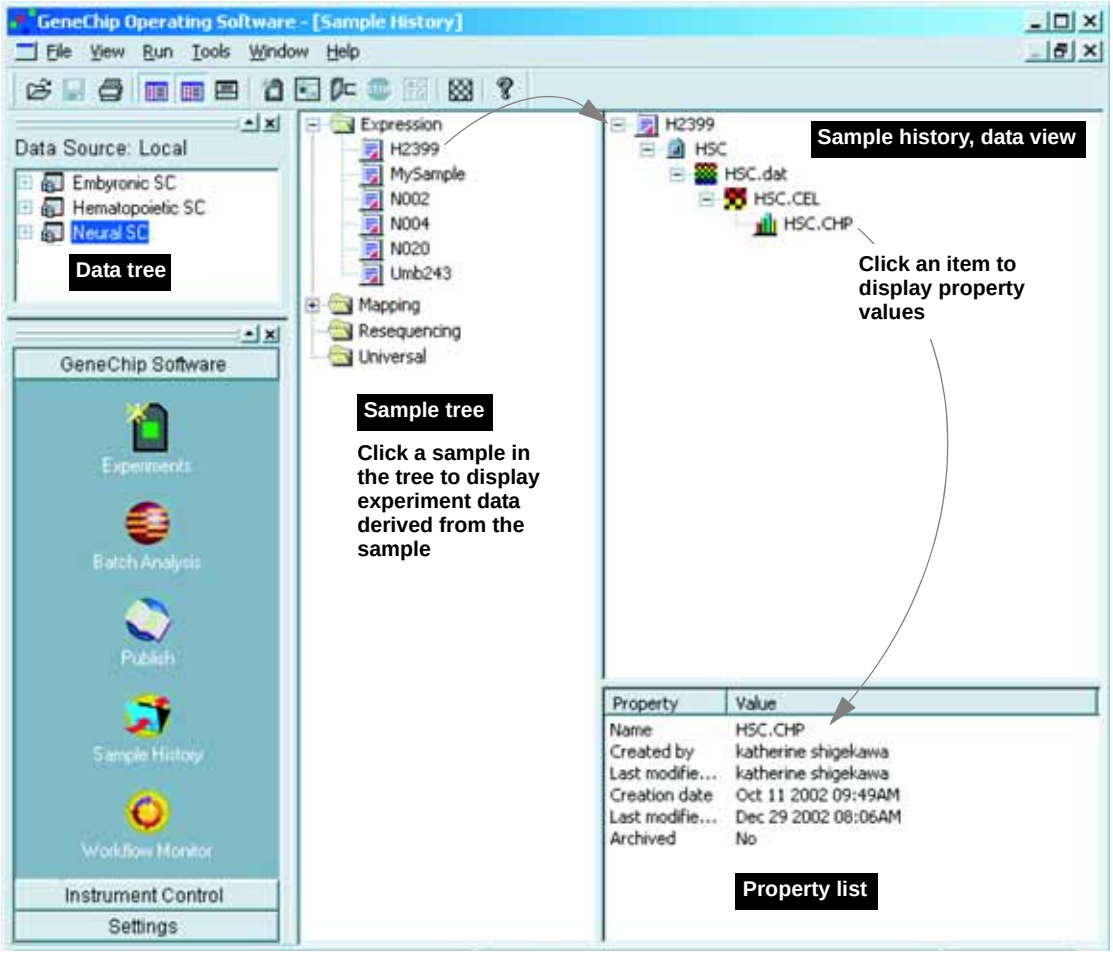


Figure 7.1
Sample history, data view

2. To display a tree of the experiment data derived from a sample, click the sample name in the sample tree.

3. To display a list of data properties and their values (bottom right pane), click an item in the sample history tree.

Displaying Sample History Process View

The process view shows all completed or pending stages for a particular sample.

1. In the GeneChip Software shortcut bar, click **Sample History** .

   ⇒ The sample history view appears.

2. To display the process view **(Figure 7.2)**, select **View →Processes** from the menu bar.

   The expression folder in the sample tree contains registered expression analysis samples.

✔ Note

If filters have been applied, not all registered samples may be displayed. See Filtering Data on page 65 for more information about filters.

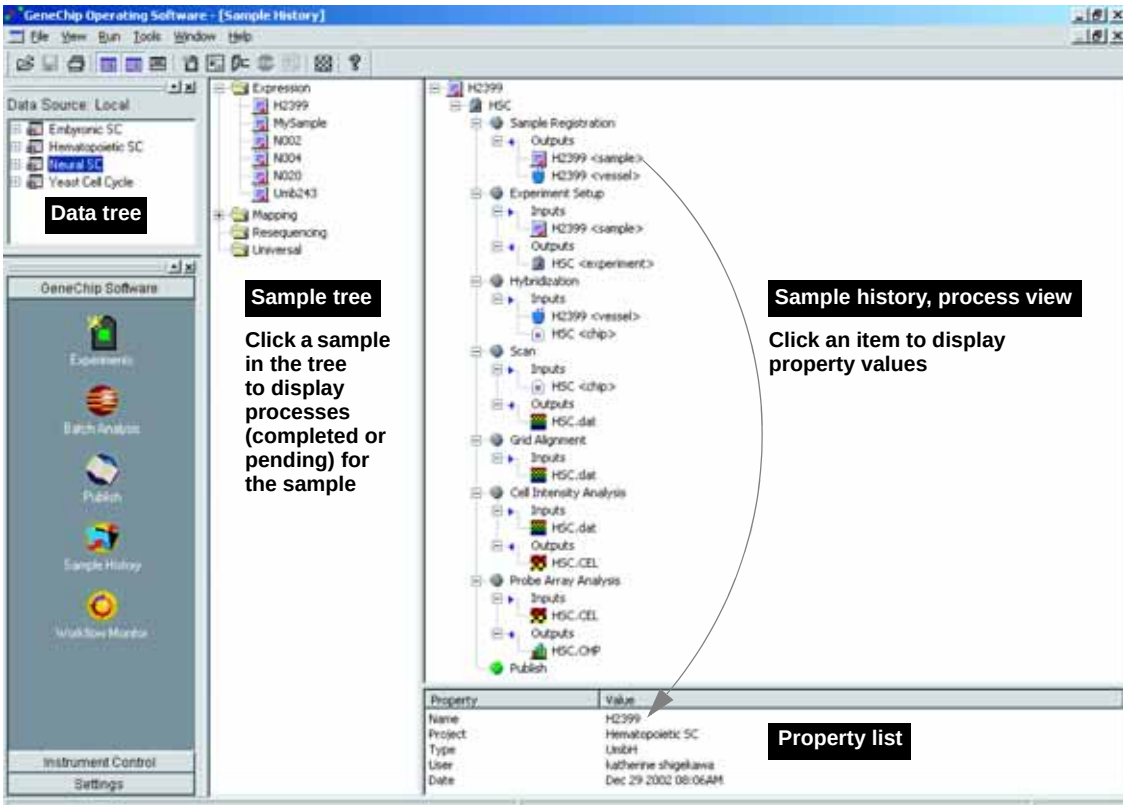


Figure 7.2
Sample history, process view

3. To display the sample history tree, click a sample name in the sample tree.

   Gray bullets indicate completed processes and green bullets indicate those that are pending.

4. Click the plus sign [+] to the left of a process name to expand the information in the sample history tree **(Figure 7.2)**. Use the scroll bars to the right or below the process tree to view the expanded information.

   The tree displays the inputs and outputs for each process. For example, cell intensity data is the input to the probe array analysis process that generates probe analysis data.

5. To display a list of data properties (bottom right pane), click one of the following in the sample history tree **(Figure 7.2)**:
   - sample name
   - experiment name
   - a process input or output

Workflow Monitor

When you define an experiment, it automatically enters the workflow and may be tracked through the sequential tabs in the workflow monitor (see **Table 7.1**).

Table 7.1
Workflow monitor tabs

Workflow Monitor Tab	Displays...
Hybridization	Experiments that have not been processed in the fluidics station.
Scan	Experiments that have been processed in the fluidics station, but have not been scanned.
Grid Alignment	Image data that require manual grid alignment.
Cell Intensity Analysis	Image data that have been manually aligned, but have not been analyzed by the cell analysis algorithm (no cell intensity data exists)
Probe Array Analysis	Cell intensity data that have not been analyzed by the expression analysis algorithm.
Publish	Expression probe array analysis data that have not been published. Note: The GeneChip® DNA Analysis Software (GDAS) handles mapping and resequencing probe array data for publishing queue purposes.

Opening the Workflow Monitor

1. In the GeneChip Software shortcut bar, click **Workflow Monitor** .
   ⇒ The workflow monitor appears **(Figure 7.3)**.

Note

If filters have been applied, not all registered samples or experimental data may be displayed. See the Filtering Data on page 65 for more information about filters.

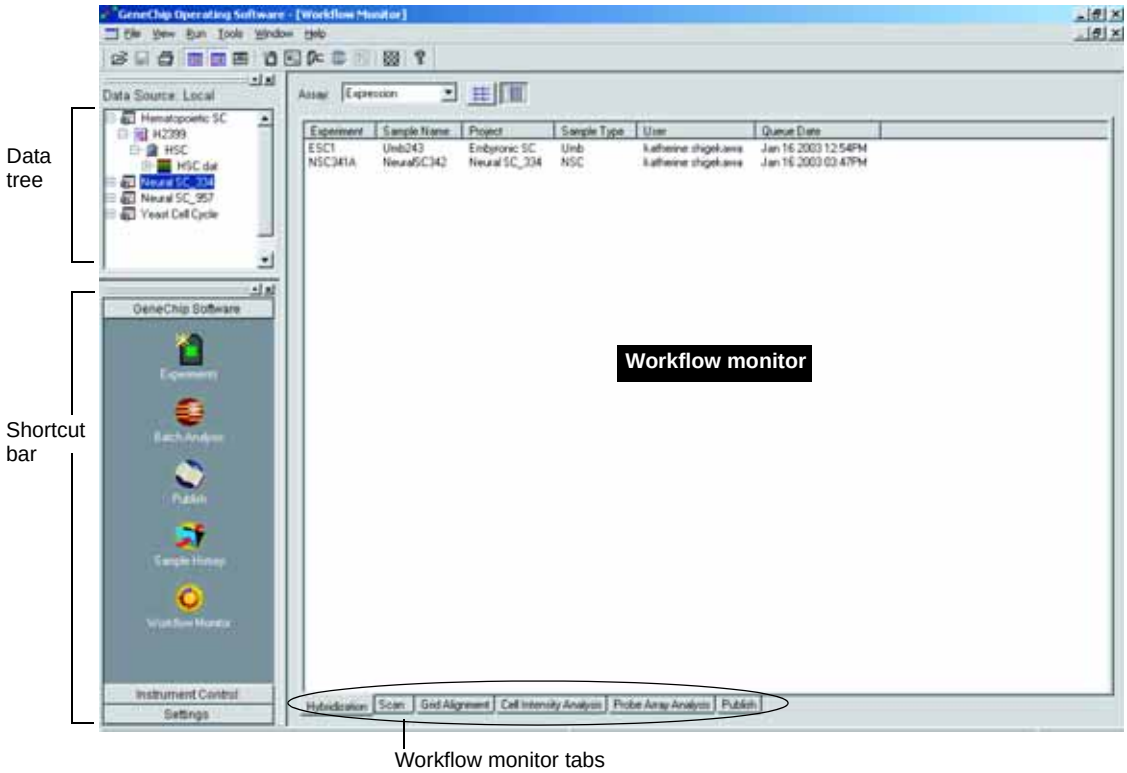


Figure 7.3
Workflow monitor, details view

2. To update the display, select **View →Refresh** from the menu bar.

   This refreshes the workflow monitor so that it displays any new items added to the system since it was last opened.

3. To view a list of experiments, click the **List** button . To view sample and project information for each experiment, click the **Details** button .

4. To view experiment or data information, right-click the item and select **Open Item** from the shortcut menu that appears. Alternatively, select **View →Open Item** from the menu bar.

Workflow Monitor Tabs

1. Click a tab to view the items in that part of the workflow queue.
2. To update the workflow monitor, select **View →Refresh** from the menu bar.

Hybridization Tab

The Hybridization tab displays experiments that have not been processed in the fluidics station. An experiment is automatically moved to the Scan tab after it has been processed in the fluidics station.

If a probe array is not processed (hybridized, or washed and stained) in the fluidics station, the experiment must be manually advanced to the Scan tab. To manually advance an experiment to the Scan tab:

- Right-click the experiment and click **Advance to Scan** in the shortcut menu that appears. Alternatively, click the experiment and select **View** → **Advance to Scan** from the menu bar.

Scan Tab

The Scan tab displays experiments that have been processed in the fluidics station, but have not been scanned. After the scan and grid alignment processes are completed, the cell intensity analysis is automatically run and the experiment is moved to the Probe Array Analysis tab. If the grid was not successfully aligned by the Alignment algorithm, the Grid Alignment tab displays the experiment.

Grid Alignment Tab

The Grid Alignment tab displays image data that require manual grid alignment.

To manually align the grid:

1. Right-click the image data in the Grid Alignment tab and click **Open Item** in the shortcut menu that appears.

   ⇒ The image is displayed.

2. Align the grid at each corner of the image:

   a. Click the ⊞ button in the Image window task bar and choose a corner.

b. Place the mouse arrow over the grid (or sub-grid) perimeter (the arrow becomes a double arrow, ↕ ⤢ ↔).

The diagonal orientation of the double arrow along the perimeter of a corner probe cell indicates horizontal and vertical adjustments can be made simultaneously using the click-and-drag method or by using the keyboard arrow keys.

c. Use the click-and-drag method or the keyboard arrow keys to adjust the horizontal or vertical position of the grid so that it is aligned over the corner of the image **(Figure 7.4)**.

3. After you align the grid at each corner of the image, click the **Save** button **(Figure 7.4)**.

⇒ The grid is saved.

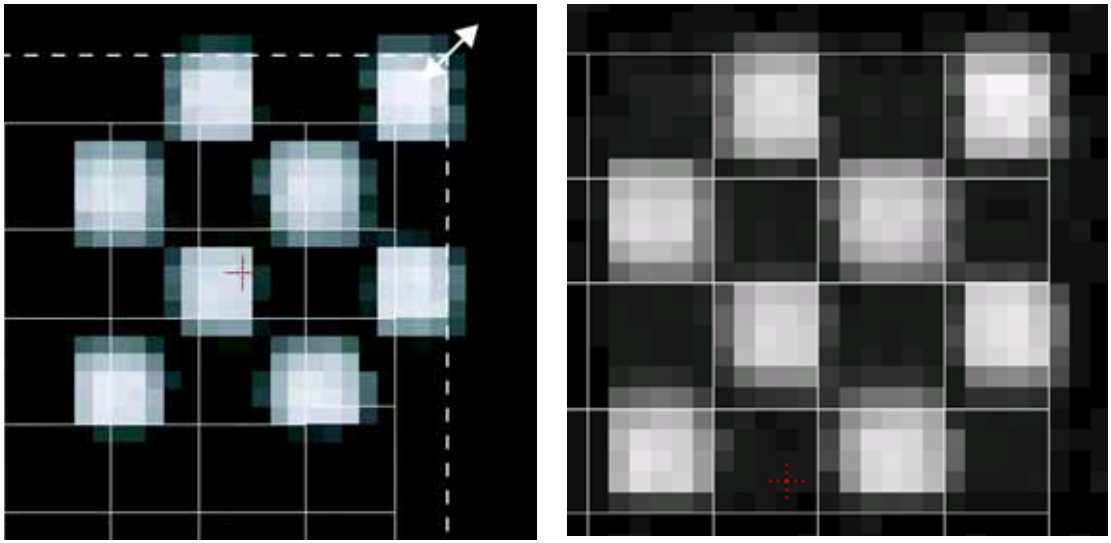


Figure 7.4
Manual grid alignment: align the grid at each corner of the image and click the **Save** button

Cell Intensity Analysis Tab

The Cell Analysis tab displays image data after the grid has been manually aligned. **GCOS does not automatically generate the cell intensity data from an image that requires manual grid alignment.** As a result, no cell intensity data exist for the image data under the Cell Analysis tab.

To generate the cell intensity data:

- In the Cell Analysis tab, right-click the image and click **Analyze Item** in the shortcut menu that appears.

  ⇒ This generates the cell intensity data and places it under the Probe Array Analysis tab.

To generate the cell intensity data and probe array analysis data:

1. In the Cell Analysis tab, right-click the image and click **Open Item** in the shortcut menu that appears.

   ⇒ This displays the image data.

2. Select **Run Analysis** from the menu bar.

   ⇒ This generates the cell intensity and probe analysis data.

   The Publish tab displays the probe analysis data.

Probe Array Analysis Tab

The Probe Array Analysis tab displays cell intensity data that have not been analyzed by the expression analysis algorithm (no probe array analysis data exists).

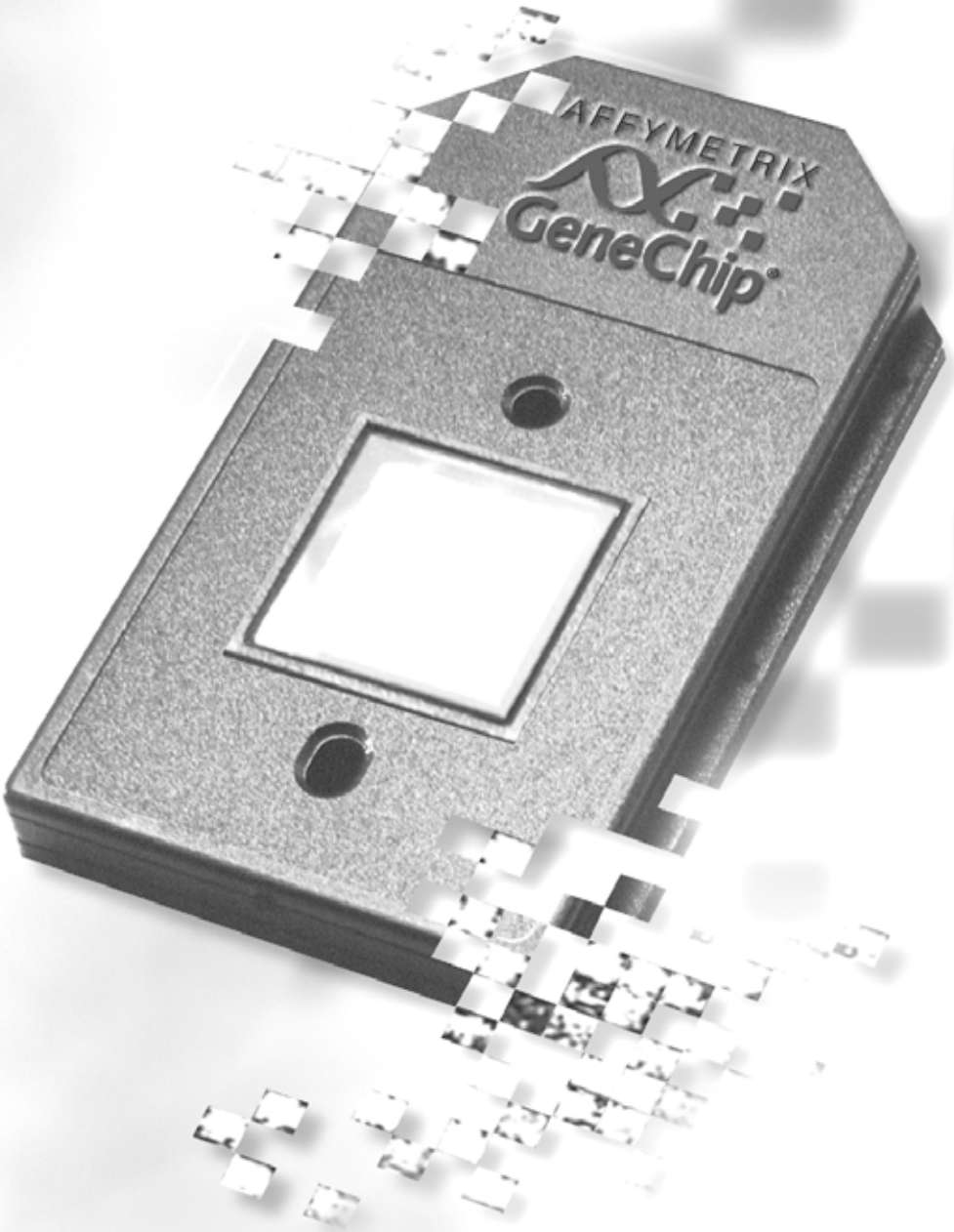
To analyze cell intensity data:

- In the Probe Array Analysis tab, right-click the cell intensity data and click **Analyze Item** in the shortcut menu that appears. Alternatively, select **View** →**Analyze Item** from the menu bar.

After the cell intensity data have been analyzed and the probe analysis data have been generated, the experiment is moved to the Publish tab.

Publish Tab

The Publish tab displays expression probe analysis data that have not been published. The probe analysis data is removed from the Publish tab and the workflow monitor after it is published.



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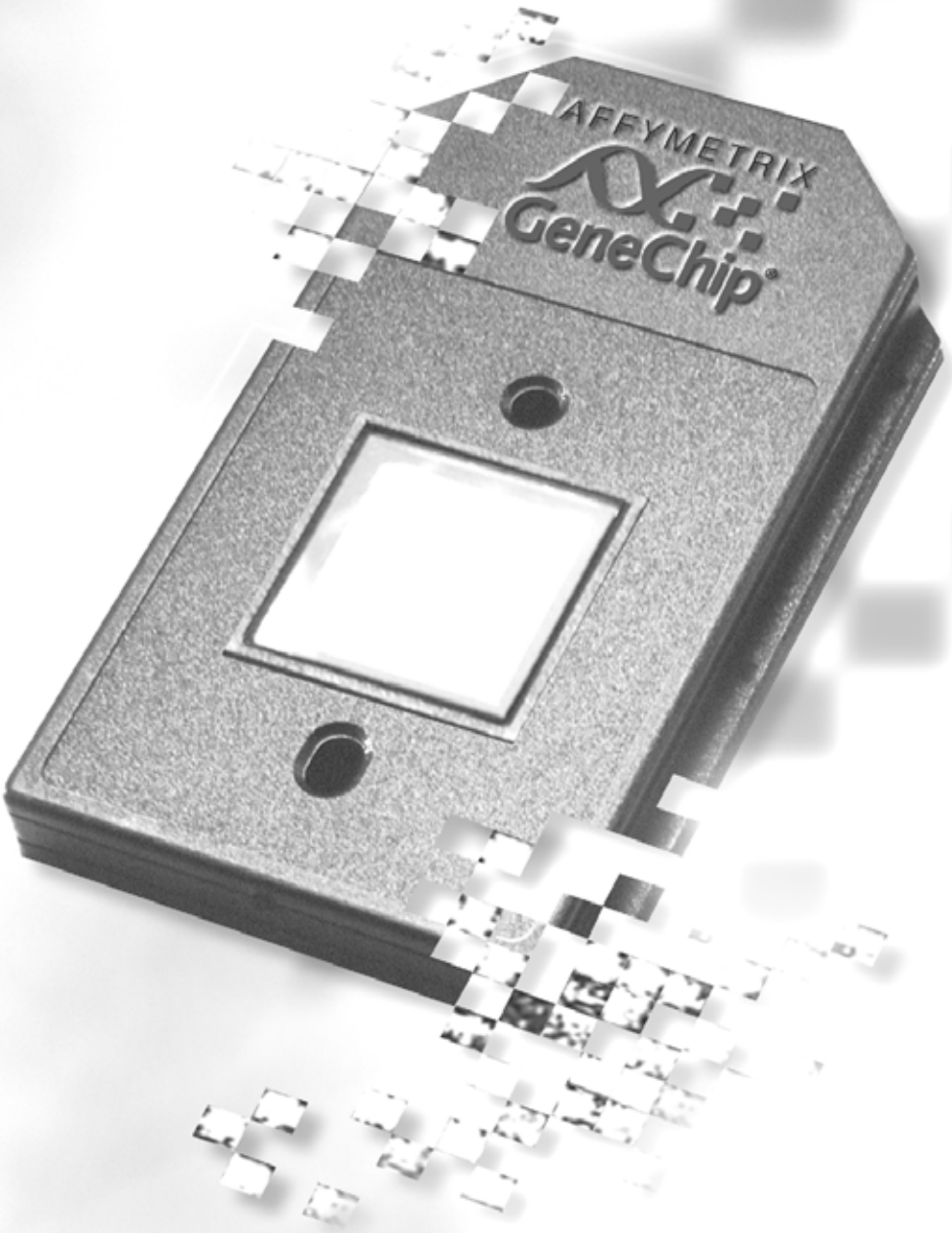


Controlling the Instruments

This chapter describes how to use the GeneChip® Operating Software (GCOS) to control the GeneChip® Fluidics Station and the Affymetrix® GeneChip® Scanner 3000 or the GeneArray® 25000 scanner **(Figure 8.1)**.

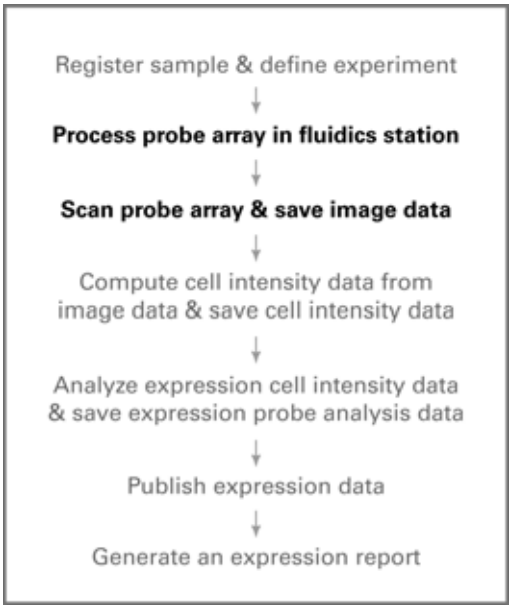


Figure 8.1
Assay & analysis flow chart

The Fluidics Station

The fluidics station hybridizes, washes, and stains the probe arrays. One workstation can control up to eight fluidics stations. Each fluidics station contains four modules and each module can independently process a probe array using a different fluidics protocol. Refer to the *GeneChip® Fluidics Station User's Guide* for a description of the instrument, its components, and set up.

✔ Note

Before you use the fluidics station, check the fluidics station configuration and prime the fluidics station with appropriate buffer. For more information, see *Configuring the Fluidics Station & Scanner*, on page 68.

Priming the Fluidics Station

Priming fills the fluidics station lines with wash buffers and deionized water. The GeneChip® Fluidics Station must be primed before it can be used to run assay protocols. Prime the fluidics station when:

- the fluidics station is first turned on
- a wash solution is changed
- the fluidics station is to be used again after a shutdown has been performed
- a module LCD window informs you that the module is not primed

1. Click the **Fluidics** button in the Instrument Control shortcut bar or the main toolbar. Alternatively, select **Run →Fluidics** from the menu bar.
   ⇒ If more than one fluidics station is installed on the workstation, the Station Selection dialog box appears **(Figure 8.2)**.
   ⇒ If only one fluidics station is installed on the workstation, the Fluidics Station dialog box appears **(Figure 8.3)**.
2. If more than one fluidics station is installed on the workstation, select the number designation of the current fluidics station from the **Station Number** drop-down list.

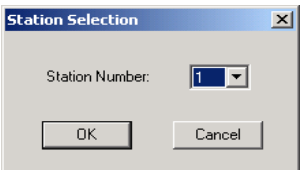


Figure 8.2
Station Selection dialog box

3. Click **OK** to close the Station Selection dialog box.

   ⇒ The Fluidics Station dialog box for the currently selected station appears **(Figure 8.3)**.

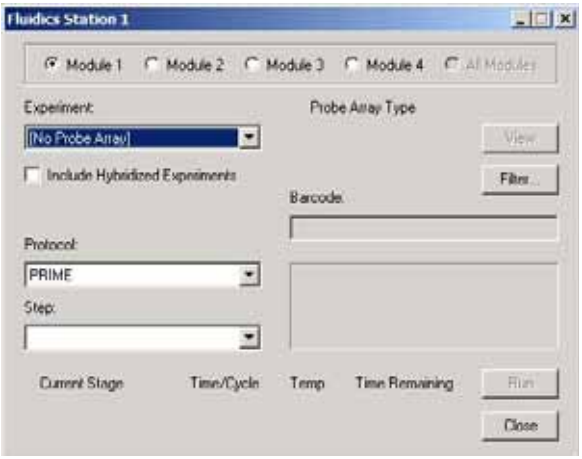


Figure 8.3
Fluidics Station dialog box, Prime protocol selected

4. Select *Prime* from the **Protocol** drop-down list and *No Probe Array* from the **Experiment** drop-down list for each module to be used.
5. Fill the intake buffer reservoirs A and B with the appropriate priming buffer. (Refer to the appropriate GeneChip® probe array package insert.)
6. Empty the waste bottle and fill the water reservoir with deionized water.

7. Load an empty, standard 1.5 mL microcentrifuge tube in the sample holder of each module to be primed.

8. Click **Run** for each module to be primed and follow the prompts in the fluidics station dialog box (also shown in the module LCD window). Alternatively, to prime all modules, choose the **All Modules** option. Click **Run** to start the priming on all modules and follow the prompts in the Fluidics Station dialog box.

   ⇒ The Fluidics Station dialog box and the module LCD window display the status of the procedure. The fluidics station is ready to use when priming is completed and **Priming done, Ready** appears in the module LCD window.

Setting Up a Fluidics Protocol

1. In the Instrument Control shortcut bar, click **Fluidics** . Alternatively, select **Run →Fluidics** from the menu bar.

   ⇒ If more than one fluidics station is installed on the workstation, the **Station Selection** dialog box appears **(Figure 8.2)**.

   ⇒ If only one fluidics station is installed on the workstation, the **Fluidics Station** dialog box appears **(Figure 8.4)**.

2. If more than one fluidics station is installed on the workstation, select the number designation of the current fluidics station from the **Station Number** drop-down list.

3. In the **Fluidics Station** dialog box **(Figure 8.4)**:

   a. Select the current fluidics station module.

   b. Choose an experiment and protocol from the drop-down lists. (Refer to the appropriate GeneChip® probe array package insert.)

   c. If a barcode was entered when the experiment was created, enter the barcode using the keyboard or scan the barcode with an external barcode reader, and press the **Tab** key. This selects the experiment associated with the barcode from the database.

4. If the selected experiment has no barcode, you can associate a barcode with the experiment by entering the barcode using the keyboard or an external barcode reader.

Note

*If filters have been applied, the **Experiment** drop-down list may not include all experiments. Click **Filter** to open the **Filters** dialog box and view or specify filters.*

*Choose the **Include Hybridized Experiments** option to include hybridized experiments in the drop-down list.*

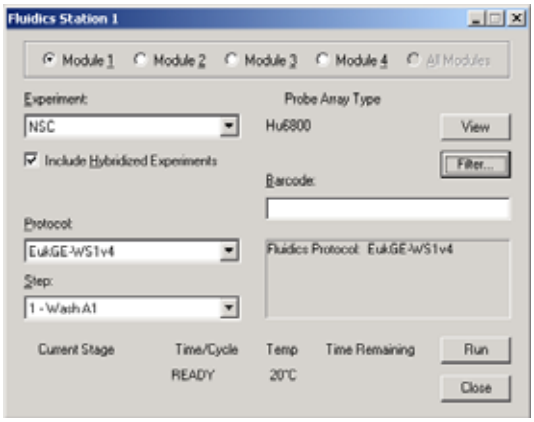


Figure 8.4
Fluidics Station dialog box

5. Click **View** to display information about the selected experiment.
6. Click **Run** to start the protocol on the selected module.
7. Repeat as necessary for other modules in the fluidics station(s).

Running the Fluidics Protocol

1. Load the probe array and sample vial holder containing the appropriate solution in each active module.

   Sensors in the fluidics station detect when the probe array and sample vial holder have been loaded. The process will proceed automatically from this point, although some protocols may require removal and substitution of the sample vial and solution. The Fluidics Station dialog box and each module LCD window display the status of the procedure.

2. After the protocol is finished, remove the probe array and inspect the probe array window for air bubbles.

If air bubbles are present, reinsert the probe array into the fluidics station to automatically drain and refill the probe array with the last wash buffer used. (Refer to the appropriate GeneChip® probe array package insert.) If no bubbles are present, the probe array is ready to be scanned.

Running a Maintenance Protocol in All Modules

The software can start a maintenance protocol in all modules of the fluidics station using a single button click. A maintenance protocol (for example, CLEAN, DRAIN, HOME, PRIME, RECOVER, SHUTDOWN, or BLEACH) does not require an experiment.

1. Select a maintenance protocol from the Protocol drop-down list.
2. Select the **All Modules** option.
3. Click **Run**.

Resuming a Fluidics Protocol

GCOS tracks the progress of a fluidics protocol run. If the protocol stops before completion, it can be resumed at the point where it was interrupted.

✔ Note

*The resume feature is only available for fluidics protocols that display multiple steps in the **Step** drop-down list of the Fluidics Station dialog box.*

If you exit GCOS while a fluidics protocol is running, the resume feature will be unavailable upon startup of GCOS.

1. When ready to resume the protocol, select the appropriate protocol from the **Protocol** drop-down list in the Fluidics Station dialog box.
2. Select the **All Modules** option.
3. Click **Run**.
   ⇒ The selected protocol is started in modules one through four of the fluidics station.

Bypassing Steps in a Fluidics Protocol

Some multi-step fluidics protocols can be started at any step, so that part of a protocol can be bypassed.

1. In the Instrument Control shortcut bar, click **Fluidics** . Alternatively, select **Run →Fluidics** from the menu bar.

   ⇒ The Fluidics Station dialog box appears **(Figure 8.5)**.

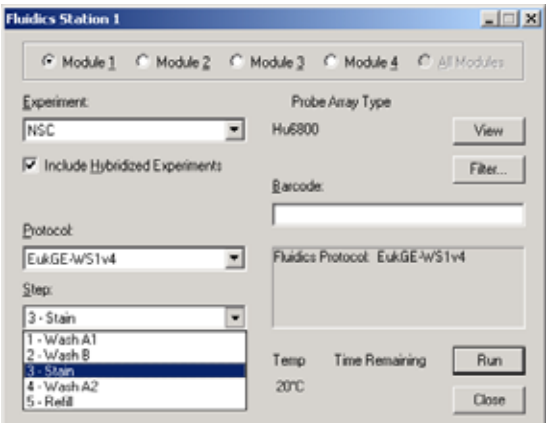


Figure 8.5
Fluidics Station dialog box, bypassing protocol steps 1 and 2

2. Select the desired experiment, module, and protocol from the drop-down lists in the Fluidics Station dialog box.

✔ Note

*The bypass function is only available for fluidics protocols that display multiple steps in the **Step** drop-down list of the Fluidics Station dialog box.*

3. Select the desired beginning step from the **Step** drop-down list **(Figure 8.5)**.

4. Click **Run** to start the fluidics protocol at the selected step.

Editing a Fluidics Protocol

You can edit some hybridization and wash (Hybwash) protocols.

✔ Note

Modifications to a Hybwash protocol must be completed before it is run. Protocol changes made during a run do not affect the run in progress.

1. Select **Tools →Edit Protocol** from the menu bar.

   ⇒ The Fluidics Protocol dialog box appears **(Figure 8.6)**.

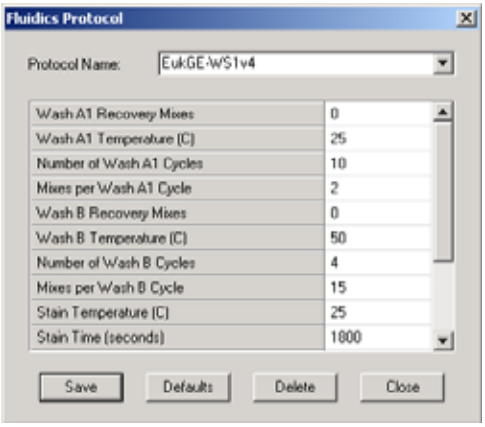


Figure 8.6
Fluidics Protocol dialog box

2. Choose the fluidics protocol you want to edit from the **Protocol Name** drop-down list.

✔ Note

Only the protocols in this list may be edited. All others are defined for specific applications and cannot be customized.

3. Highlight the parameter value you want to change and enter the new value. (Parameters values must be within the ranges in **Table 8.1**.) Enter a Hybridization Time of zero if only a wash is desired. To omit Wash A or B, enter zero for the Number of Wash A or Wash B cycles.

Table 8.1
Valid ranges for hybridization or stain protocol parameters

Parameter	Valid Range
Hybridization or stain time	0 - 86,399 seconds
Temperature	15 - 50° C
Number of Wash cycles	0 - 99
Mixes per Wash cycle	1 - 99

4. To save the parameters under the same protocol name (overwrites the old protocol), click **Save**.
5. To save the parameters under a new protocol name, enter a new name in the **Protocol Name** field, then click **Save**.
   ⇒ This adds the new protocol name to the drop-down list.
6. Click **Defaults** to return the parameter settings to the default values.
7. Click **Delete** to delete the currently selected protocol from the system.

Editing the Fluidics ID Associated with a Fluidics Station

GCOS has the ability to determine the fluidics station identity (ID) and module number used to wash and stain a probe array. The local or GCOS server database keeps track of the fluidics station ID and the module number that processes an experiment (probe array).

To enable this feature, you must configure the registry using the regedit application so that GCOS can read the fluidics station ID and module number information from the registry.

1. Click the Windows **Start** menu button

and select **Run**.
   ⇒ The Run box appears **(Figure 8.7)**.

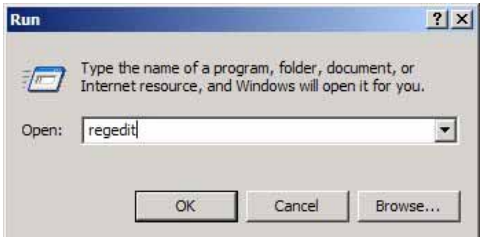


Figure 8.7
Run box

2. Enter *regedit* at the command prompt and click **OK**.

   ⇒ The Registry Editor appears **(Figure 8.8)**.

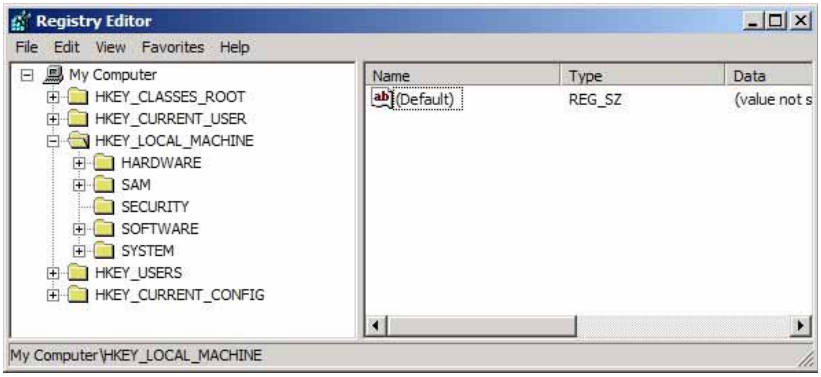


Figure 8.8
Registry Editor

3. Open the HKEY_LOCAL_MACHINE registry hive.

4. Select the **Software →Affymetrix →GeneChip →Fluidics** node under the HKEY_LOCAL_MACHINE registry hive **(Figure 8.9)**.

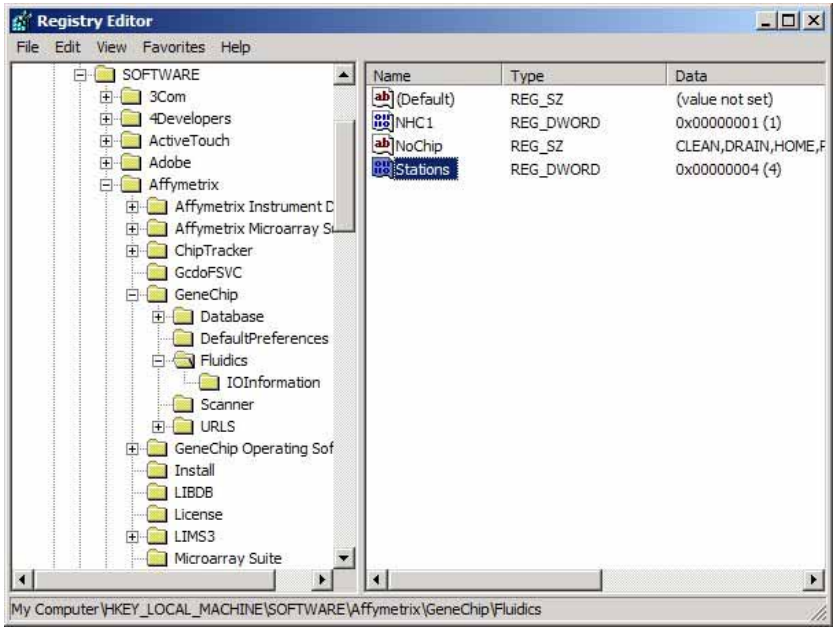


Figure 8.9
Registry Editor

5. In the right window, right-click and select **New →DWORD Value** from the shortcut menu to create a new name/data pair. Repeat for each fluidics station whose ID is to be captured **(Figure 8.10)**.

✔ Note

GCOS allows a maximum of eight fluidics stations to be controlled by a single workstation.

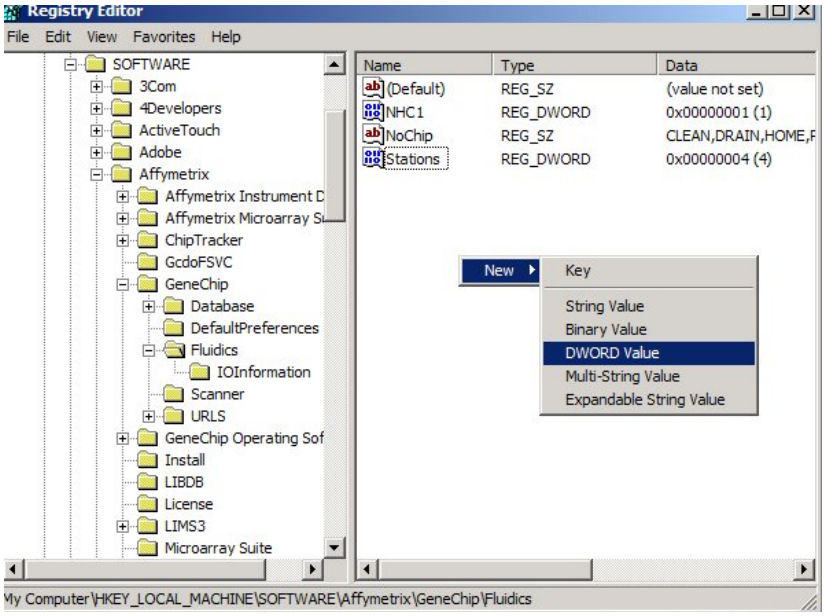


Figure 8.10
Registry Editor. create new name/data pair

6. Select the first newly created Name/Data pair, right-click the name, and select **Rename** from the shortcut menu. Rename the entry to “ID1”.

   The example in **Figure 8.11** shows four Name/Data pairs that have been renamed ID1, ID2, ID3, and ID4.

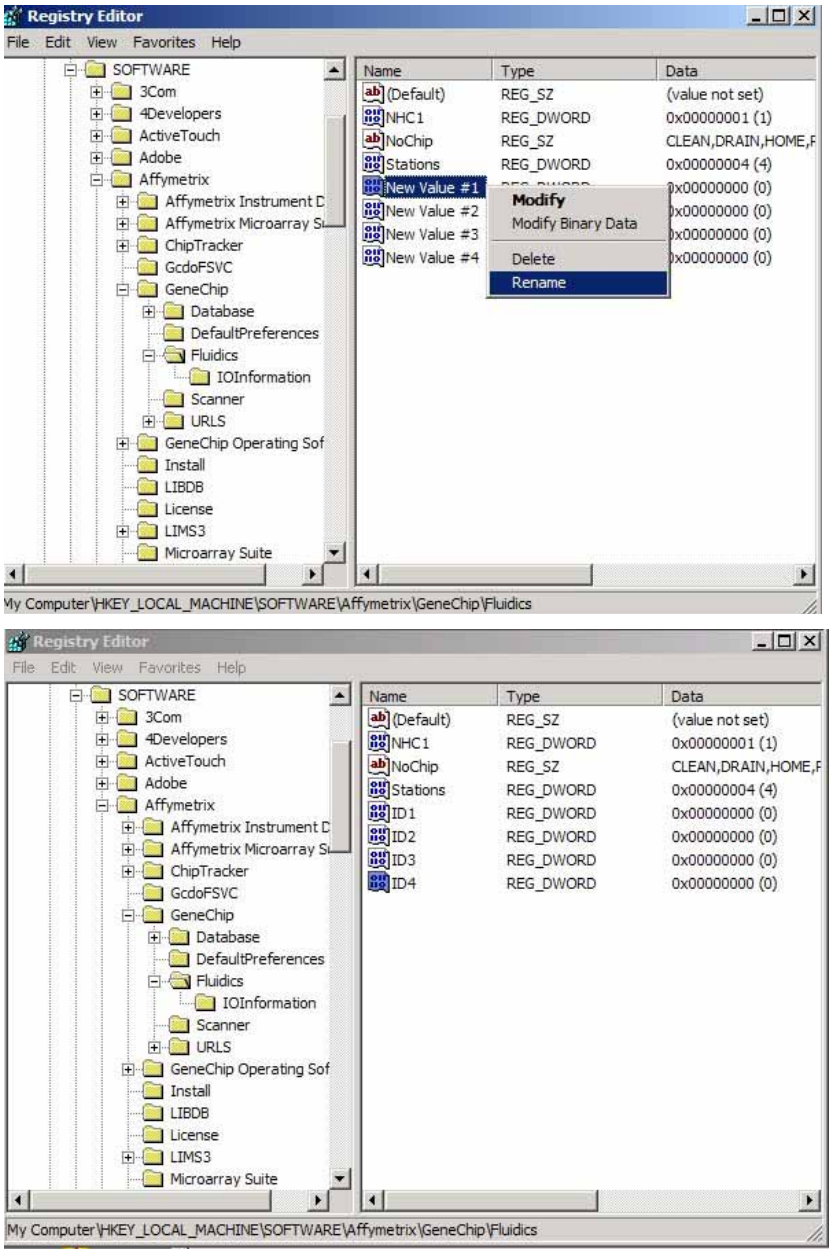


Figure 8.11
Registry Editor, four newly created Name/Data pairs renamed ID1, ID2, ID3, and ID4

7. To edit the value of an entry, double-click the entry.

   ⇒ The Edit DWORD Value box appears **(Figure 8.12)**.

Figure 8.12
Edit DWORD Value box

8. Select the Decimal option and enter a new value for the station ID.

9. Repeat step 7 and 8 to edit the value of each entry.

   In this example, stations one through four have the ID 1001, 1002, 1003, and 1004 respectively **(Figure 8.13)**. When a fluidics protocol is run on any of these stations, the fluidics station ID and the module number will be tracked in the process database.

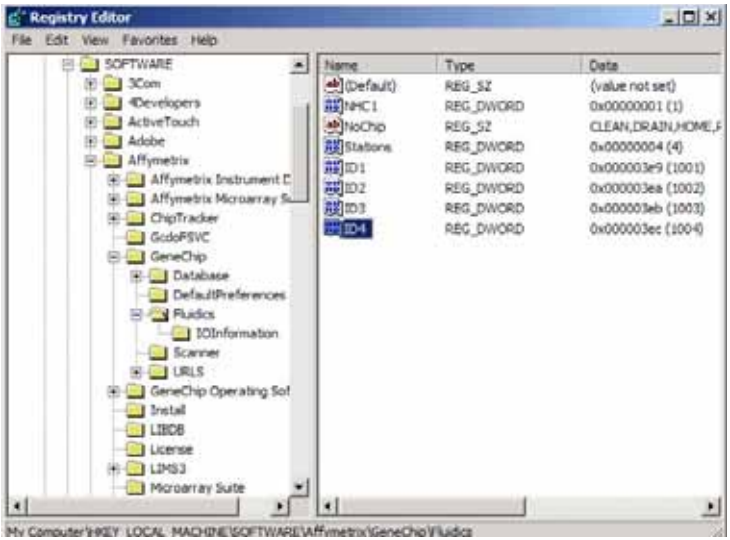


Figure 8.13
Registry Editor

Scanning a Probe Array– GeneChip® Scanner 3000

This section shows you how to scan a GeneChip® probe array using the Affymetrix® GeneChip® Scanner 3000. Make sure to read and understand Appendix D, *Using the GeneChip® Scanner 3000*, on page 491 before you scan a probe array.

Using Tough-Spots™ to Prevent Leaks

Tough-Spots™ are chemically inert polyvinyl labels that adhere to all plastics. Affymetrix recommends using 3/8-inch circle diameter Tough-Spots to prevent leakage from the cartridge septa.

Before loading the probe array cartridge, follow this procedure to prevent the leaking of fluids from the cartridge during scanning.

Even if you have already applied Tough-Spots to the cartridge prior to hybridization or after washing, you must remove the old Tough-Spots and apply new ones before you load them into the AutoLoader.

> Affymetrix recommends the use of Tough-Spots™ obtained from Affymetrix P/N 64-0158
>
> or from
>
> USA Scientific, Inc. P.O. Box 3565 Ocala, FL 34478 (800)LAB-TIPS P/N 9185-0000

IMPORTANT *To reduce the risk of leakage, do not use excessively large pipette tips to pierce the septa.*

1. On the back of the probe array cartridge, clean excess fluid from around septa **(Figure 8.14)**.

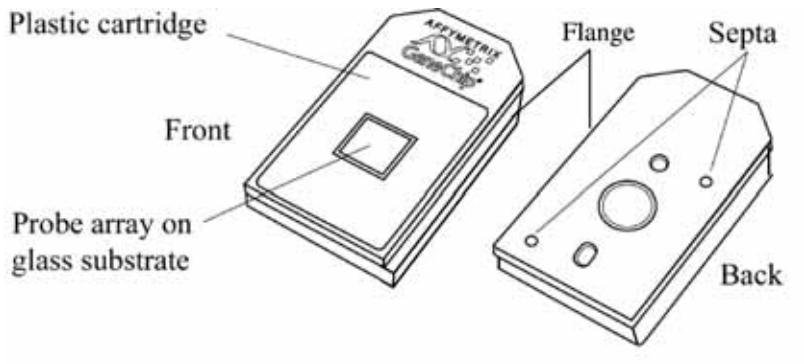


Figure 8.14
The GeneChip® probe array cartidge

2. Carefully apply one Tough-Spot over each of the two septa. Press to ensure that the spots remain flat. If a Tough-Spot does not apply smoothly; that is, if you observe bumps, bubbles, tears or curled edges, do not attempt to smooth them out. Remove the spot and apply a new one **(Figure 8.15)**.

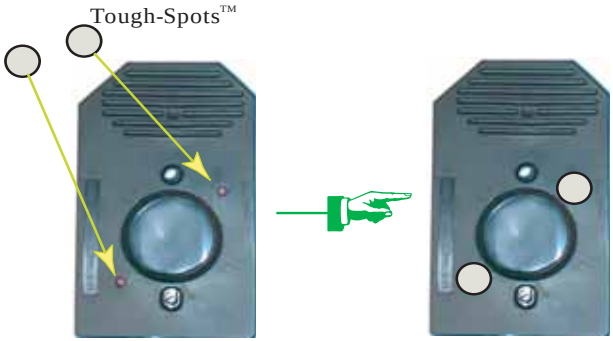


Figure 8.15
Applying Tough-Spots™ to cartridge septa

Scanning a Probe Array

1. Press the on/off (I/O) switch on the front panel. The scanner's on board computer will boot up. The bootup process takes a couple of minutes. During this time both the yellow and green lights will be on.

2. Click the **Start Scanner** button in the Instrument Control shortcut bar or the main toolbar. Alternatively, select **Run →Start Scanner** from the menu bar.

   ⇒ The Scanner dialog box appears **(Figure 8.16)**. Note that the dialog also displays barcode information if you entered it when you created the experiment or set up the fluidics protocol.

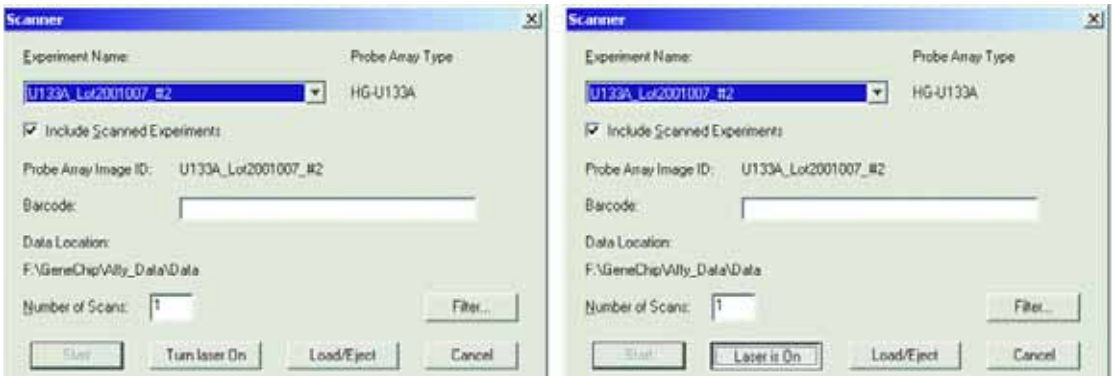


Figure 8.16
Scanner dialog box, laser off (left) and laser on (right)

3. To include scanned experiments in the **Experiment Name** drop-down list, choose the **Include Scanned Experiments** option.

   By default, the **Experiment Name** drop-down list displays the experiments in the current directory that have not been scanned (no image data exists for the experiments).

✔ Note

*If filters have been applied, the drop-down list may not include all experiments. Click **Filter** to open the **Filters** dialog box and view or specify filters.*

4. Select the experiment name of the probe array to be scanned from the **Experiment Name** drop-down list. Alternatively, set the focus to the

barcode field. If a barcode was associated with the experiment to be scanned, scan or manually enter the barcode from the probe array. This automatically retrieves the experiment from the database.

The **Probe Array Type** field automatically displays the probe array type that was entered during experiment setup. If a barcode was entered when the experiment was created or hybridized, the barcode field displays the barcode.

GCOS sets the **Number of Scans** per probe array type (generally one for the Scanner 3000). You may change the number of scans in the **Scanner** dialog box **(Figure 8.16)**. Multiple scans are statistically averaged to create the image data.

✔ Note

Increasing the number of scans increases the scan time as well as the amount of fluorophore bleaching and may result in lower fluorescence intensities.

5. If it is necessary to rescan a probe array, select the experiment name from the **Experiment Name** drop-down list. Also select **Include Scanned Experiments**.

IMPORTANT

*The existing image data will be overwritten when the probe array is rescanned. To retain the original scan data, use the **File →Save As** command from the menu bar to save the original image data under a different name.*

6. Click **Start** in the **Scanner** dialog box.

✔ Note

*When the software displays the **Laser is On** button in the scanner dialog box, the **Start** button is activated and you can start a scan.*

⇒ The GeneChip Scanner dialog box appears **(Figure 8.17)**.

Figure 8.17
Scanner dialog box

⇒ The scanner door opens and the chip transport mechanism raises to accept a probe array.

7. Load the probe array **(Figure 8.18)** into the scanner chip transport mechanism. Insert the probe array into the chip transport mechanism such that the front of the probe array (label side) faces to the rear of the scanner **(Figure 8.19)**.

✔ Note

The scanner will enter Park mode if it is unattended for 15 minutes and will enter standby, or sleep, mode if it is unattended for 60 minutes (45 minutes after entering Park mode). The green light will turn off and the yellow light will turn on. To reactivate the laser, click ***Turn Laser On*** *in the scanner dialog box* **(Figure 8.16)***and wait 10 minutes.*

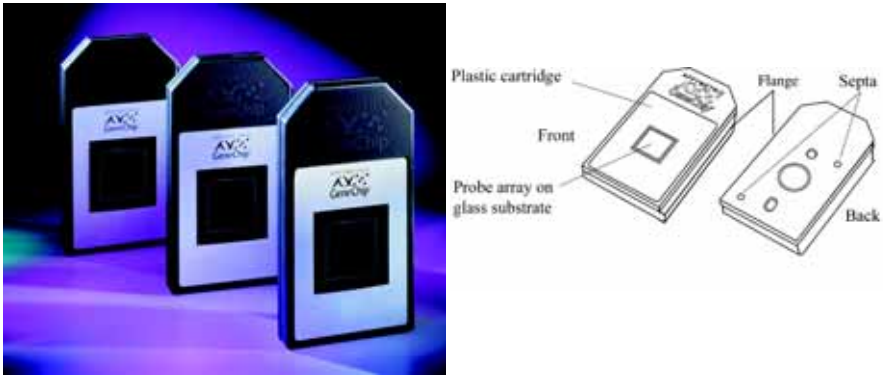


Figure 8.18
Affymetrix® GeneChip® probe arrays: note the location of the flange. The scanner will accept the probe array in only one orientation.

Figure 8.19
Loading the probe array into the chip transport mechanism. Note that the front of the probe array faces to the rear of the scanner.

! CAUTION

Do not force the probe array cartridge. If the cartridge does not drop easily into the chip transport mechanism, eject the cartridge and try again. If this does not remedy the situation, see Troubleshooting on page 504 or call Affymetrix technical support.

If a probe array becomes lodged in the scanner, you can manually remove it. See Issues Relating to the Scanner's Operation on page 505.

To view the scanner options, click **Options** in the GeneChip Scanner dialog box **(Figure 8.20)**.

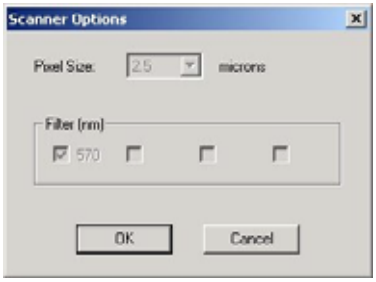


Figure 8.20
Scanner options dialog box. You cannot change these options. This is for information only.

✔ Note

You cannot modify the scanner settings. GCOS automatically selects the appropriate settings based on the probe array type specified during experiment setup.

8. Click **OK** in the GeneChip Scanner dialog box to start scanning the probe array.

   During the scan, the green light will flash, and the yellow light will be off.

   If the scan in progress feature is enabled (select **View →Scan in Progress** from the menu bar), the Image window automatically opens in the main display area when a scan starts. It displays the fluorescence intensity of the probe array at approximately 50 lines at a time as the scan progresses.

   To enable (or disable) this option, select **View** from the menu bar and place (or remove) a check mark next to **Scan in Progress**.

   After the scan is completed, GCOS:

   - saves the image data (displayed in the main display area)
   - aligns a grid on the image to identify the probe cells
   - automatically computes probe cell intensities and generates the cell intensity data
   - ejects the probe array

✔ Note

If you leave the scanner idle for an additional 15 minutes, the scanner will also enter "Park" mode. The yellow light will be off and the green light on.The chip transport mechanism will retract and the scanner door will close. You must click the **Eject Chip** *button to open the scanner door and raise the chip transport mechanism.*

Stopping a Scan

1. Click the **STOP** button or select **Run →Stop Scanner** from the menu bar.
2. At the prompt, click **Yes** to stop the scanner or **No** to cancel stopping **(Figure 8.21)**.

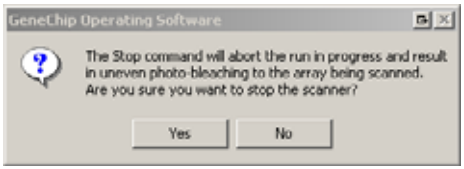


Figure 8.21
Stop scanner prompt

! CAUTION

If you click Yes, the data from a partial scan will be lost. This is different from using earlier software or scanner versions where you could save the data from a partial scan.

If you rescan a probe array that has been partially scanned, the previously scanned area of the probe array may experience fluorophore bleaching. This will result in non-uniform fluorescence intensity across the probe array.

3. After you stop a scan, the scanner will automatically eject the chip.

✔ Note

The scanner dialog box (**Figure 8.16** on page 113) *has an eject chip button. This is reserved for ejecting a chip after the scanner goes into sleep mode or when you must manually eject the chip for any other reason.*

If the probe array cartridge becomes stuck, see Troubleshooting on page 504 or call Affymetrix technical support.

Shutting Down the Scanner

1. Close the GCOS software.
2. Press the I/O (on/off) button on the front panel to turn off the instrument.

✔ Note

The laser also has a sleep mode that activates after 1 hour of inactivity.

Scanning a Probe Array– GeneArray® 2500 Scanner

GCOS controls the GeneArray® 2500 scanner and enables you to initiate a scan of a GeneChip® probe array, view the image data as they are collected during scanning, and analyze the data after the scan is completed. Refer to the *Agilent GeneArray Scanner User's Guide* for a description of the instrument, its components, and set up.

! CAUTION

For Windows 2000, Affymetrix recommends that you boot up the workstation first, log into the workstation, then turn the GeneArray® 2500 scanner on and launch GCOS.

If you do not follow this sequence, the GeneArray® 2500 scanner may freeze and show the red LED. If this occurs, turn off then turn on the scanner.

This does not affect the Affymetrix GeneChip® Scanner 3000.

Scanning a Probe Array

1. Click the **Start** button in the Instrument Control shortcut bar or the main toolbar. Alternatively, select **Run →Start Scanner** from the menu bar.

   ⇒ The Scanner dialog box appears **(Figure 8.22)**.

IMPORTANT

Do not attempt to scan until the laser has completed its warmup period (15 minutes).

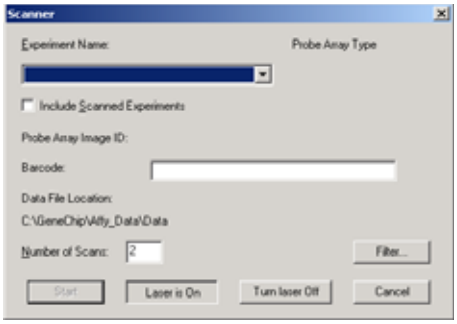


Figure 8.22
Scanner dialog box

The **Experiment Name** drop-down list displays the experiments in the current directory that have not been scanned (no image data exists for the experiments).

Note

*If filters have been applied, the drop-down list may not include all experiments. Click **Filter** to open the **Filters** dialog box and view or specify filters.*

After the scanner completes a scan, GCOS saves the scanned image data.

2. To include scanned experiments in the **Experiment Name** drop-down list, choose the **Include Scanned Experiments** option.

3. Select the experiment name of the probe array to be scanned from the **Experiment Name** drop-down list. Alternatively, set the focus to the barcode field. If a barcode was associated with the experiment to be scanned, scan or manually enter the barcode from the probe array. This automatically retrieves the experiment from the database.

   When you have choosen the experiment name, the Start button will highlight.

   The **Probe Array Type** field automatically displays the probe array type that was entered during experiment setup. If a barcode was entered when the experiment was created or hybridized, the barcode field displays the barcode.

   GCOS sets the **Number of Scans** according to the probe array type (generally two for the GeneArray® scanner). You may change the number of scans in the **Scanner** dialog box **(Figure 8.22)**. Multiple scans are combined to create the image data.

Note

Increasing the number of scans increases the scan time as well as the amount of fluorophore bleaching and may result in lower fluorescence intensities.

4. If it is necessary to rescan a probe array, select the experiment name from the **Experiment Name** drop-down list.

IMPORTANT

*The existing image data will be overwritten when the probe array is rescanned. To retain the original scan data, use the **File →Save As** command from the menu bar to save the original image data under a different name.*

5. Click **Start** in the **Scanner** dialog box.

   ⇒ The GeneChip Scanner options window appears **(Figure 8.23)**.

Figure 8.23
Scanner options window

6. Load the probe array in the scanner and close the scanner door **Figure 8.24** and **Figure 8.25**).

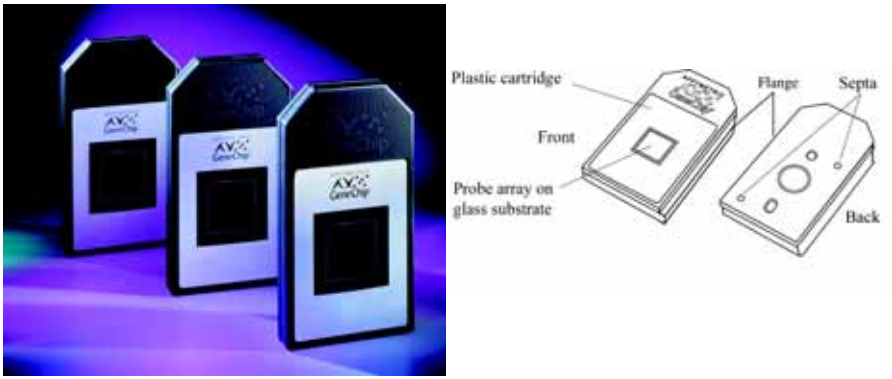


Figure 8.24
Affymetrix® GeneChip® probe arrays: note the location of the flange. The scanner will accept the probe array in only one orientation.

Figure 8.25
Loading the probe array into the GeneArray scanner.

7. To view the scanner options, click **Options** in the GeneChip Scanner options window **(Figure 8.23)**.

   ⇒ The Scanner Options dialog box appears **(Figure 8.26)**.

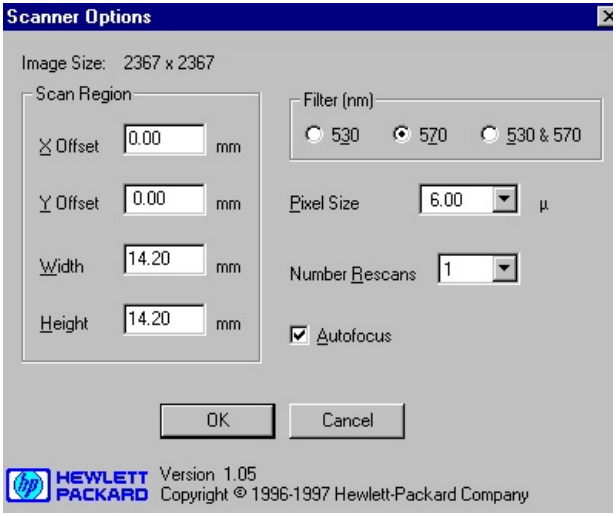


Figure 8.26
Scanner options dialog box

Note

Do not modify the scanner settings. GCOS automatically selects the appropriate settings based on the probe array type specified during experiment setup.

8. Click **OK** to close the Scanner Options dialog box.

9. Click **OK** in the GeneChip Scanner dialog box to start scanning the probe array.

   If the view scan in progress feature is enabled (select **View →Scan in Progress** from the menu bar), the Image window automatically opens in the main display area when a scan starts. It displays the fluorescence intensity of the probe array line by line as the scan progresses.

   To enable (or disable) this option, select **View** from the menu bar and place (or remove) a check mark next to **Scan in Progress**.

   After the scan is completed, GCOS:

   - saves the image data (displayed in the main display area)
   - aligns a grid on the image to identify the probe cells

- automatically computes probe cell intensities and generates the cell intensity data

Stopping a Scan

1. Click the **STOP** button or select **Run** →**Stop Scanner** from the menu bar.
2. At the prompt, click **Yes** to stop the scanner or **No** to cancel stopping **(Figure 8.27)**.

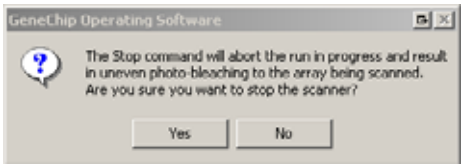


Figure 8.27
Stop scanner prompt

Note

*Selecting the same experiment name and repeating a scan of the probe array overwrites the image data of a partial scan that was saved. To retain the original scan data, use the **File** →**Save As** commands from the menu bar to save the image data under a different name prior to starting another scan of the probe array.*

Shutting Down the Scanner

1. Click the **Scanner** button or select **Run** →**Start Scanner** from the menu bar.

   ⇒ The Scanner dialog box appears **(Figure 8.28)**.
2. Click **Turn laser Off** in the Scanner dialog box.

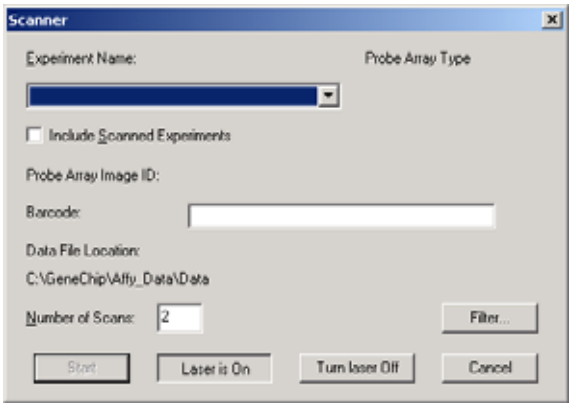


Figure 8.28
Scanner dialog box

3. If the Stop Scanner prompt appears, click **Yes** to turn off the laser power immediately and start the laser cool down routine.

   The laser cool down routine lasts for several minutes and is finished when the cooling fan in the laser power supply shuts down. After the cooling fan shuts down, you can safely turn off the scanner power switch.

Editing the Scanner ID Associated with the GeneArray® Scanner

GCOS can determine the identity (ID) of the scanner used to scan a probe array. The local or GCOS server database tracks the scanner ID for an experiment (probe array). This feature is automatically enabled when using the GeneChip® 3000 scanner.

To enable this feature for the GeneArray® scanner, you must configure the registry using the regedit application so that GCOS can read the scanner ID information from the registry.

1. Click the Windows **Start** menu button and select **Run**.

   ⇒ The Run box appears **(Figure 8.29)**.

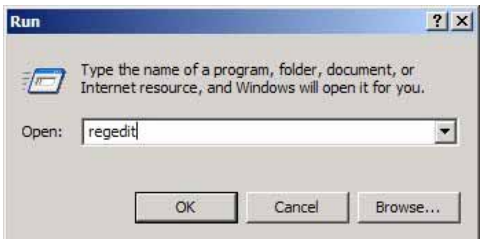


Figure 8.29
Run box

2. Enter *regedit* at the command prompt and click **OK**.

   ⇒ The Registry Editor appears **(Figure 8.30)**.

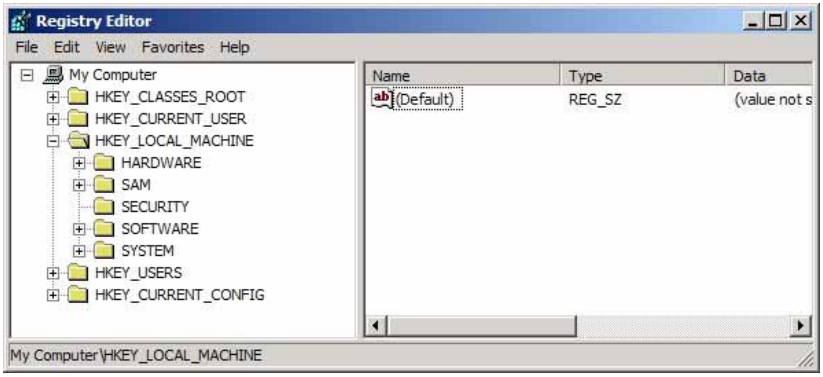


Figure 8.30
Registry Editor

3. Open the HKEY_LOCAL_MACHINE registry hive.

4. Select the **Software →Affymetrix →GeneChip →Scanner** node under the HKEY_LOCAL_MACHINE registry hive **(Figure 8.31)**.

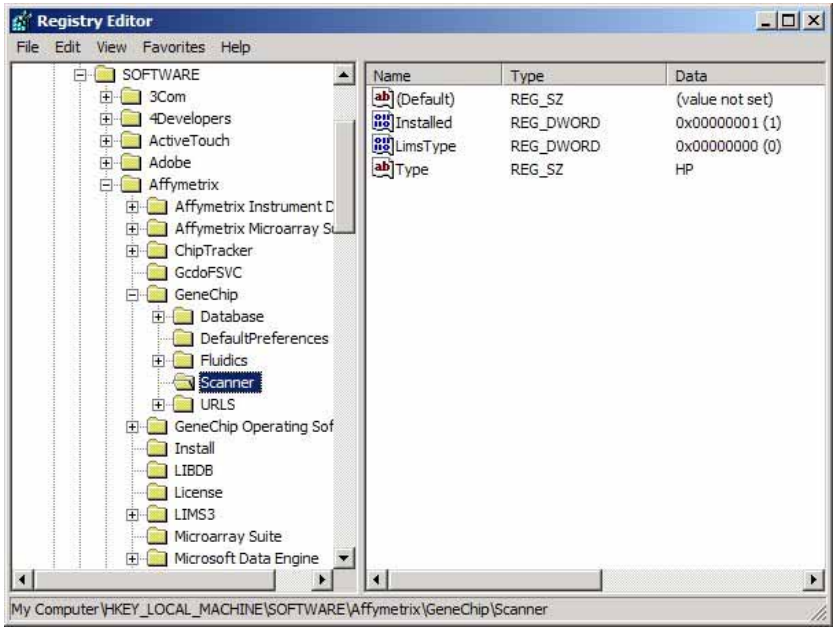


Figure 8.31
Registry Editor

5. In the right window, right-click and select **New →String Value** from the shortcut menu to create a new name/data pair **(Figure 8.10)**.

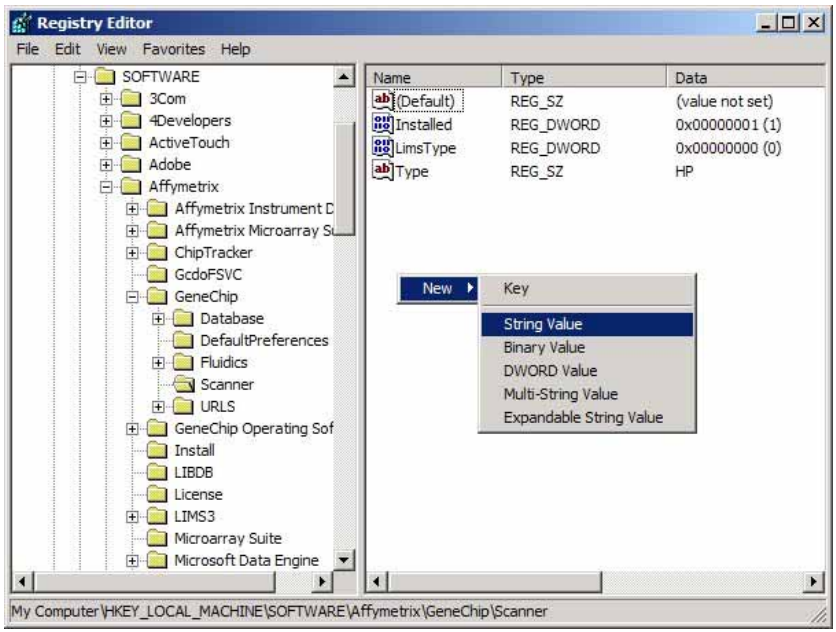
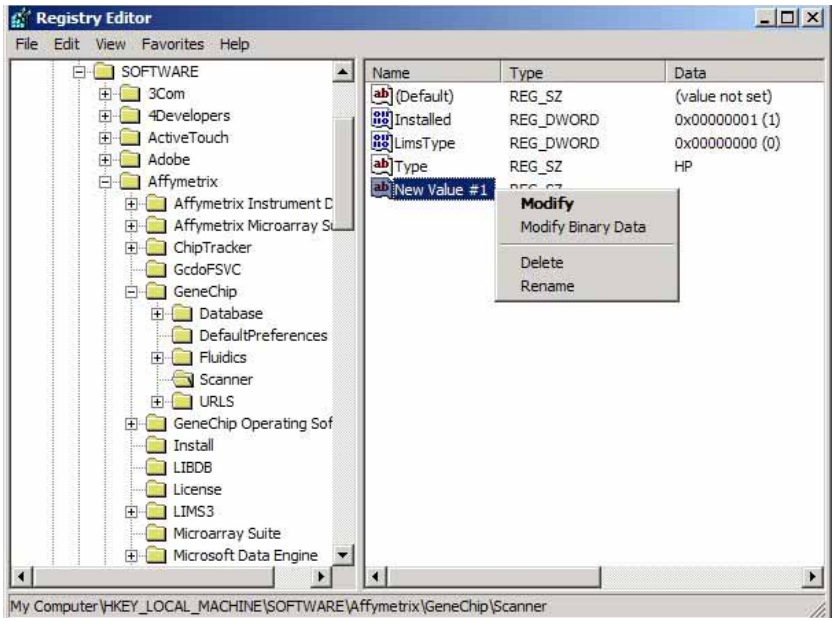


Figure 8.32
Registry Editor

6. Right-click the newly created Name/Data pair and select **Rename** from the shortcut menu. Rename the entry to "ID" as shown in **Figure 8.33**.



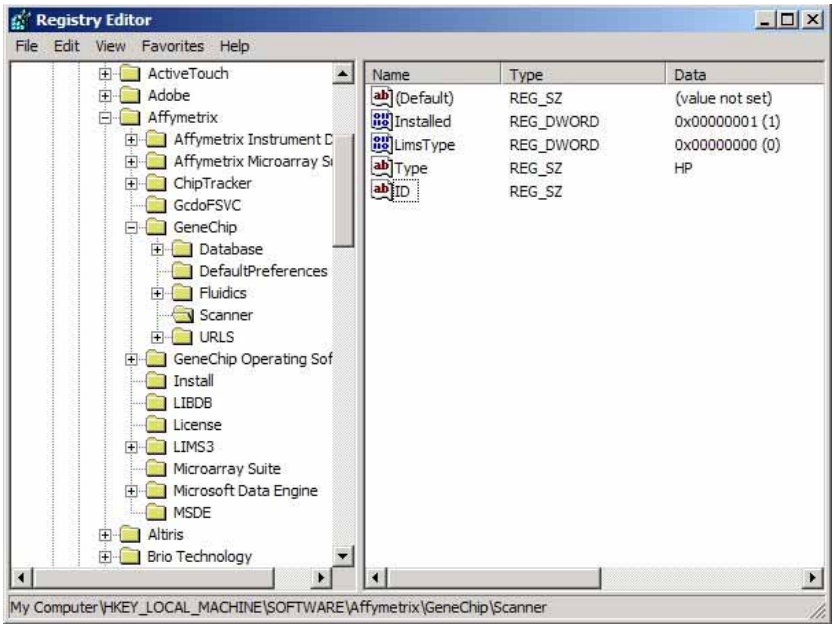


Figure 8.33
Registry Editor, newly created Name/Data pair renamed "ID"

7. To edit the value of the entry, double-click the entry.

   ⇒ The Edit String box appears **(Figure 8.34)**.

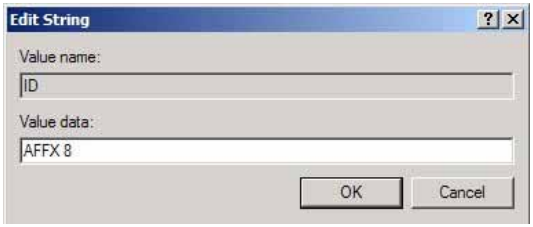


Figure 8.34
Edit String box

8. Rename the ID to a small string value of less than eight characters and click **OK**.

   In this example, the scanner ID is renamed to AFFX8 **(Figure 8.35)**. When an experiment is scanned on this scanner, the scanner ID will be tracked in the process database.

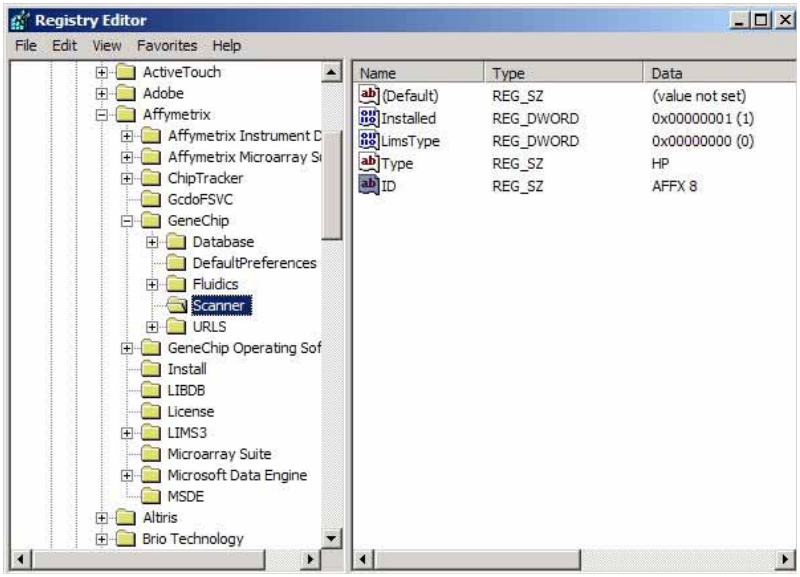
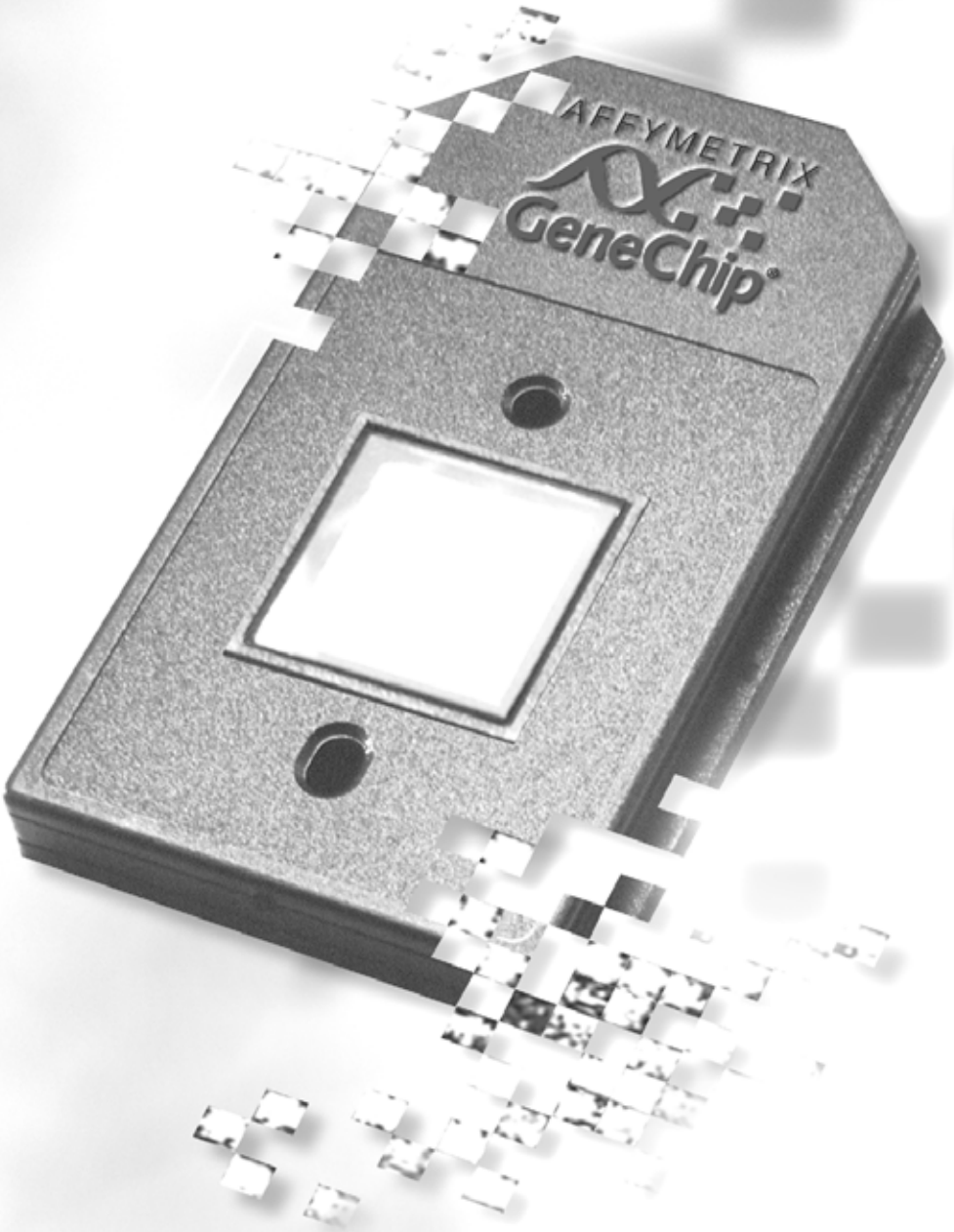
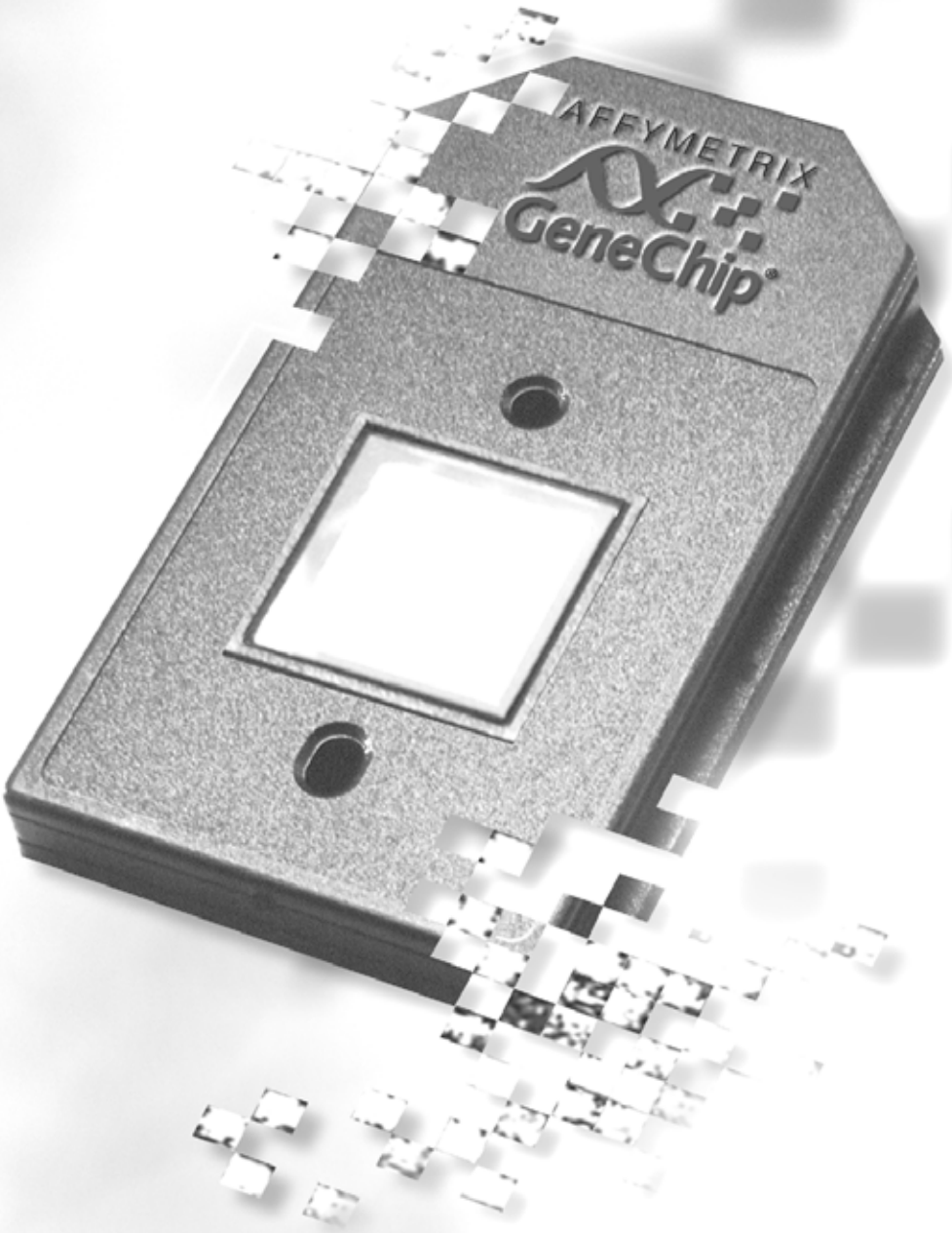


Figure 8.35
Registry Editor



9

Chapter 9



Working with Images

This chapter explains how to view the scanned image or cell intensity data in the Image window. In the Image window, you can:

- adjust image settings for the image or cell intensity data
- view data for a user specified probe cell
- highlight user specified tiles on the probe array
- create bookmarks in the image data or cell intensity data
- view and mask probe cell outliers

The Image Window

The Image window **(Figure 9.1)** displays scanned image data or the computed cell intensity data.

The Image window opens when:

- a probe array is scanned and the **Scan in Progress** feature is enabled See the section on page 108 or *Scanning a Probe Array–GeneArray® 2500 Scanner* on page 120.
- image data or cell intensity data are open (double-click the item in the data tree or click the **Open** button . Alternatively, select **File** →**Open** from the menu bar to view the data in the current directory).

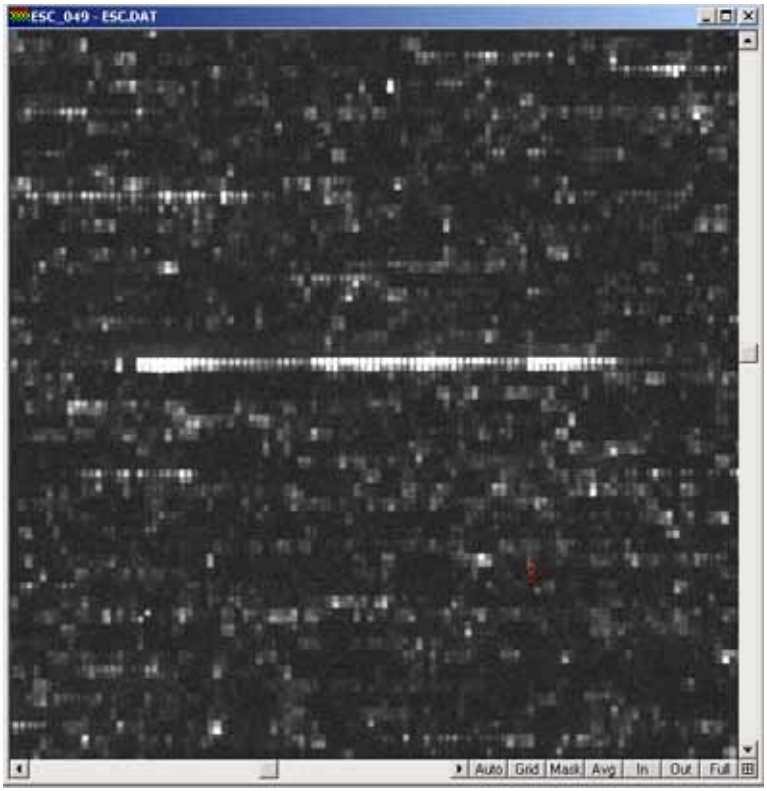


Image window task bar

Figure 9.1
Image data in the Image window

The Image window displays the image data or cell intensity data name (same as the experiment name entered during experiment setup) in the upper left corner.

- Use the scroll bars at the bottom and right side of the Image window to navigate the scan image.

Commands in the Image Window Task Bar

Auto Automatically scales the intensity of the current image based on the range of intensity levels in a selected region of the scan image. See *Adjusting the Image Settings* on page 143.

Grid Toggles the grid on and off the scan image. (Press the **G** key.) See *Viewing the Grid* on page 138.

Mask Masks user selected areas of the scan image. Masked probe cells are excluded from the analysis. See *Outliers* on page 159.

Avg Calculates the average intensity and standard deviation for a user selected region of the scan image. See *Calculating Average Intensity for User Specified Probe Cells in a Scan Image* on page 143.

In Incrementally zooms in on the scan image. Press the **I** key. Alternatively, use a click-and-drag operation to draw a rectangle over the area of interest, then click **In** to zoom directly to the selected area.

Out Zooms out incrementally from the scan image (press the **O** key).

Full Zooms out completely to the full scan image view (press the **Shift+O** keys).

⊞ Enables the user to select and zoom directly to each corner of the scan image to check grid alignment. See *Manually Aligning a Grid* on page 139.

Opening Multiple Image Windows

You can open and view more than one Image window in the main display area.

1. In the data tree, double-click the image data or cell intensity data that you want to view.

   This displays each data type in the main display area.

2. Select **Window →Cascade** from the menu bar to display an overlapping cascade of the open Image windows. Select **Window →Tile** to display the open Image windows side by side **(Figure 9.2)**.

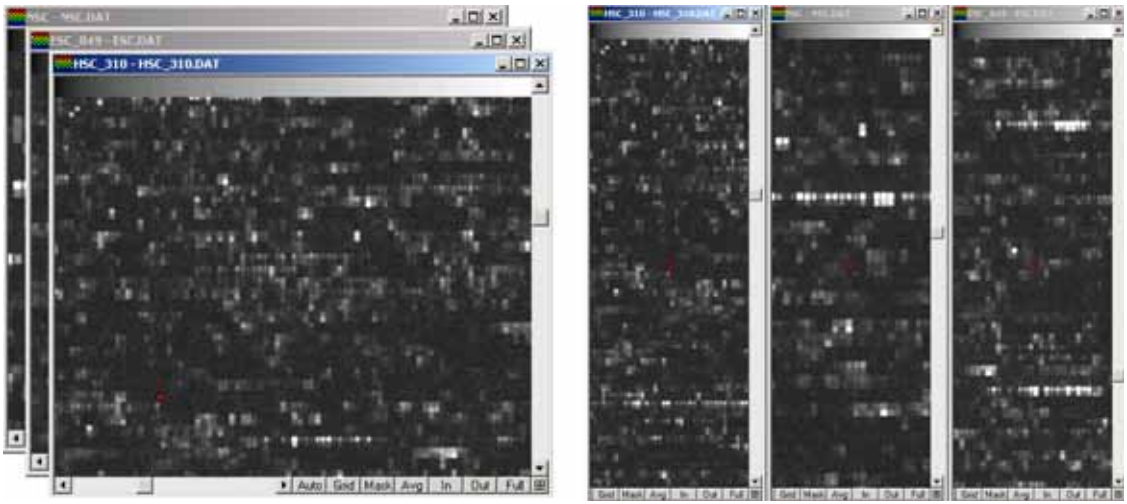


Figure 9.2
Multiple image windows, cascade view (left) and tile view (right)

3. If other data types are open (for example, an experiment or probe analysis data) and displayed in the main display area, click **Image Views** in the GeneChip Software shortcut bar to display the open Image windows.

Working With the Grid

The Alignment algorithm uses the checkerboard image of the control probes located at the corners of the probe array to superimpose a grid on the scan image. The algorithm aligns the grid so that each square in the grid delineates a probe cell.

For a CustomSeq™ probe array, the algorithm aligns a grid composed of sub-grids on the scan image from. The position of each sub-grid can be adjusted independent of the other sub-grids.

Viewing the Grid

1. To toggle the grid on or off, do one of the following:
   - click **Grid** in the Image window task bar **(Figure 9.1)**

- press the **G** key
- select **View** →**Grid** from the menu bar

2. To confirm the grid is properly aligned, do one of the following to check each corner of the scan image close up:
   - click the ⊞ button in the Image window task bar and choose a corner
   - press **F5** (upper left corner), **F6** (upper right corner), **F7** (lower left corner), or **F8** (lower right corner)
   - select **View** →**Corner** from the menu bar and choose the corner you want to view

Manually Aligning a Grid

On a rare occasion the grid may be misaligned and require manual adjustment. GCOS notifies you if manual adjustment is required.

1. Align the grid at each corner of the image:
   a. Click the ⊞ button in the Image window task bar and choose a corner.
   b. Place the mouse arrow over the grid perimeter (the arrow becomes a double arrow, ↕ ⤢ ↔).
      The diagonal orientation of the double arrow along the perimeter of a corner probe cell indicates horizontal and vertical adjustments can be made simultaneously using the click-and-drag method or by using the keyboard arrow keys.
   c. Use the click-and-drag method or the keyboard arrow keys to adjust the horizontal or vertical position of the grid so that it is aligned over the corner of the image **(Figure 9.3)**.

2. After you align the grid, click the **Save** button or select **File** →**Save** from the menu bar.

   ⇒ The grid is aligned.

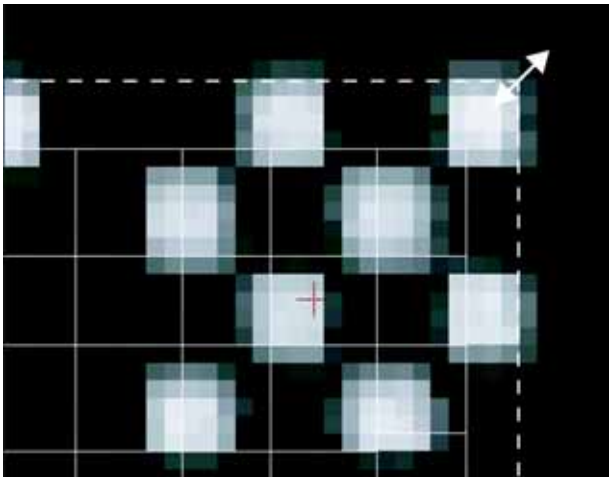


Figure 9.3
Manual grid alignment: align the grid at each corner of the image

Computing Cell Intensities

GCOS automatically generates the cell intensity data from the image data **(Figure 9.5)**. The Cell Analysis algorithm analyzes the image data and computes a single intensity value for each probe cell on the array and saves the cell intensity data.

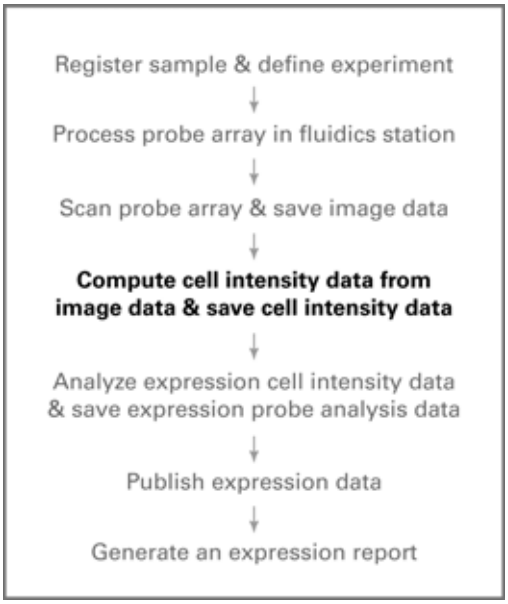


Figure 9.4
Assay & analysis flow chart

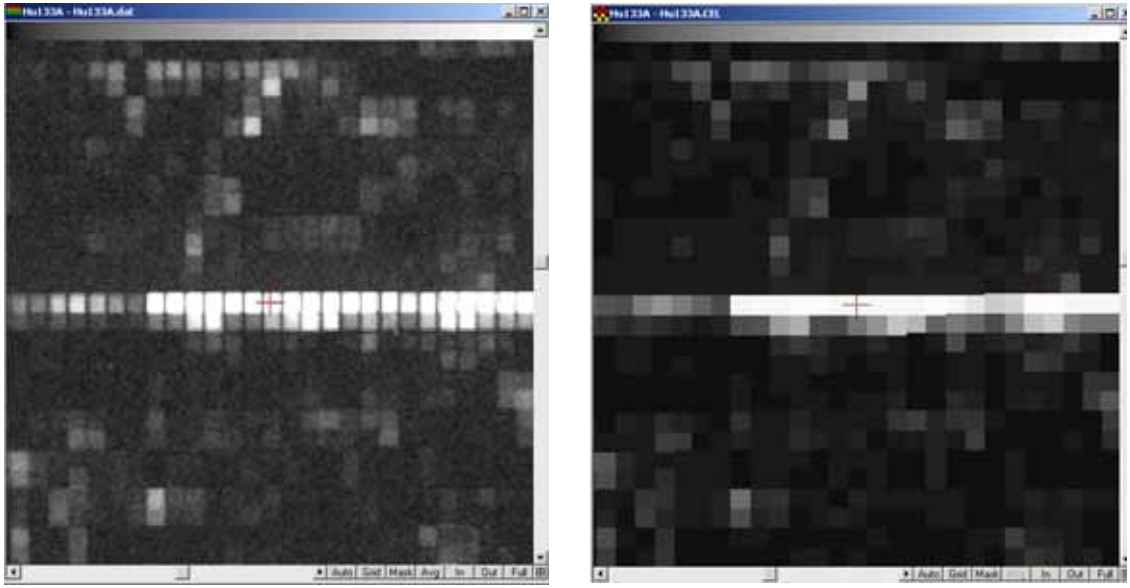


Figure 9.5
Image window, scan image data (left) and computed cell intensity data (right)

Reanalyzing Image Data

GCOS automatically generates the cell intensity data from the image data. In some cases you may want to generate new cell intensity data (for example, if the grid has been realigned).

To reanalyze image data, right-click the open image and select **Cell Grids** → **Recalculate Cell Intensity** from the shortcut menu that appears.

Viewing a Scan Image or Cell Intensity Data

Calculating Average Intensity for User Specified Probe Cells in a Scan Image

GCOS can calculate the average intensity for a user-specified group of probe cells in the image data.

1. In the image, use the click-and-drag method to select the probe cells of interest.
   ⇒ The selected probe cells are outlined in the image.
2. Click **AVG** in the Image window task bar (**Figure 9.1** on page 136).
   ⇒ The status bar displays the average intensity and the standard deviation.
3. Position the mouse arrow inside the selected area of the image.
   ⇒ A pop-up tool tip displays the average intensity and the standard deviation for the selected group of probe cells **(Figure 9.6)**.

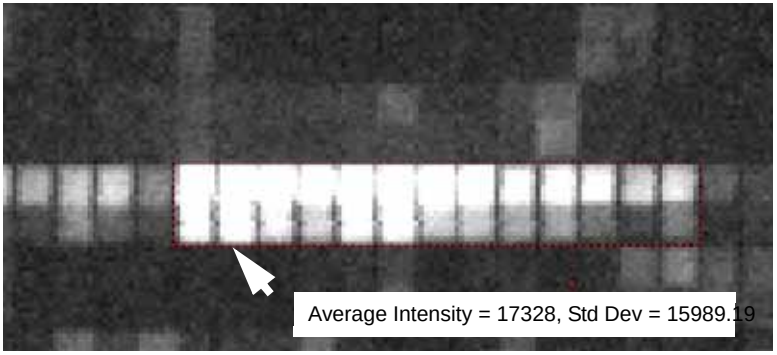


Figure 9.6
Average intensity and standard deviation for user-specified probe cells in the image data (.dat)

Adjusting the Image Settings

You can adjust image settings (intensity range, color, coordinates, and highlights) for image or cell intensity data. The image settings may be adjusted for the current data only or globally for all subsequently opened image or cell intensity data.

Making Adjustments to the Current Image or Cell Intensity Data Only

- To adjust the settings for the current image or intensity data only, do one of the following:
  - Click the **Image settings** button .
  - Press the **S** key.
  - Select **Edit** →**Image Settings** from the menu bar.

  ⇒ This displays the **Image Settings** dialog box for the active image or cell intensity data **(Figure 9.7)**.

✔ Note

All settings (except the color setting) in the Image Settings dialog box apply only to the current image or cell intensity data and do not affect other image or intensity data. The settings (except for the color setting) are not saved after you close the image or intensity data.

Click the **S** key

Select **Tools** →**Defaults** from the menu bar

Settings for the active image or cell intensity data only.

Default settings for subsequently opened image or cell intensity data

Figure 9.7
Image Settings dialog box for the active image or cell intensity data (left); Defaults dialog box, Image Settings tab (right)

Making Global Adjustments to Subsequently a Opened Image or Cell Intensity data

- To adjust settings globally, select **Tool** →**Defaults** from the menu bar.

  ⇒ This displays the Image Settings tab of the Defaults dialog box **(Figure 9.7)**.

✔ Note

The settings specified in the Image Settings tab of the Defaults dialog box do not affect open image or cell intensity data, but are applied to all subsequently opened image or cell intensity data. These default image settings are saved when the image is closed.

Setting the Intensity Range

GCOS records a dynamic intensity range from 0 to 65,000 and provides 256 colors from black through white for image display. The intensity range for image or cell intensity data is divided into 256 bins and each bin is assigned a color or gray scale.

You may enter a new lower or upper limit for the intensity range associated with image or cell intensity data and apply this subset of the dynamic range to the image. Lowering the upper limit increases the image brightness and raising the lower limit decreases the brightness. This process is called *image scaling* and can be used to adjust the display of image or cell intensity data for optimum viewing. Alternatively, GCOS can automatically scale the image using the minimum and maximum pixel intensities of the image.

1. To autoscale the current image, choose the **Autoscale** option in the Image Settings dialog box **(Figure 9.7)**
2. Click **OK** to automatically scale the image.

   When the **Image Settings** dialog box is reopened, it displays the minimum and maximum pixel intensities used to scale the image.
3. To return the intensity settings (autoscale option and intensity range) to the defaults in the Defaults dialog box, click **Defaults** in the Image Settings dialog box.

Selecting Color Settings

- To display the image or cell intensity data in:
  - **Gray scale** - choose the **Gray** color option **(Figure 9.7)**.
  - **Pseudo color** - choose the **Pseudo Color** option.

    ⇒ This applies rainbow colors to the intensity scale.
- To display a gray scale or pseudo color bar at the top of the Image window, choose the **Color Bar** option.

Selecting Display Coordinates

- Click the image to display image coordinates.

  ⇒ A red cross hairs marks the location.

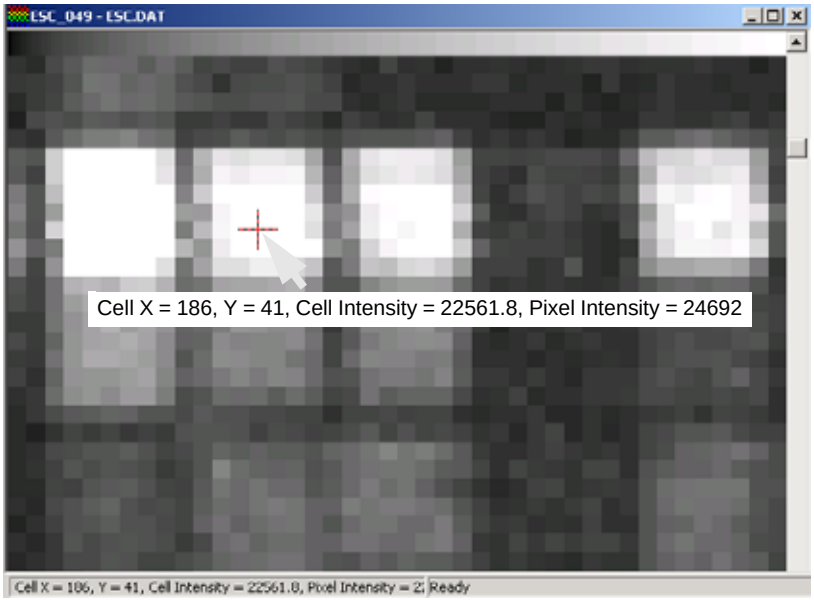
  The status bar (lower left in the main window) displays the intensity data and x,y coordinates of a probe cell or pixel indicated by the cross hairs.

If you choose the **Cell** option in the Image Settings dialog box **(Figure 9.7)**, the status bar and a pop-up tool tip display the x,y coordinates of the cell, the cell intensity, and the pixel intensity **(Figure 9.8)**.

If you choose the **Pixel** option, the pop-up tool tip and the status bar display the x,y coordinates of the pixel and the pixel intensity **(Figure 9.8)**.

✔ Note

Only the Cell option is available for cell intensity data.



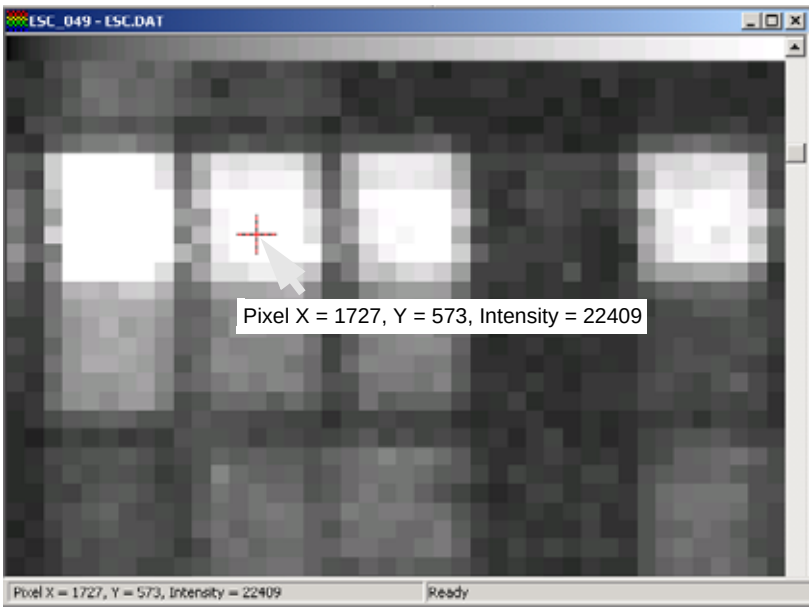


Figure 9.8
Image data, probe cell coordinates (top) and pixel coordinates (bottom)

Specifying Highlight Colors

You may specify new highlighting colors for the grid, masked cells, outlier cells, or highlighted probe array cells (features).

1. Click **Highlights** in the Image Settings dialog box (**Figure 9.7** on page 144)**.**

   ⇒ The **Highlight Colors** dialog box appears **(Figure 9.9)**.

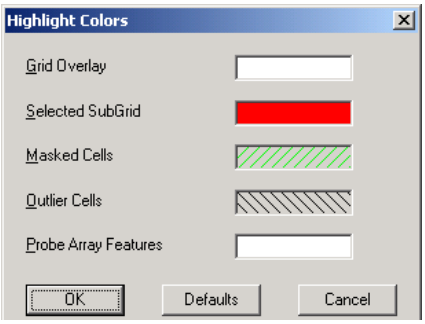


Figure 9.9
Highlight Colors dialog box

2. To change the color of a particular item (for example, the grid overlay):

   a. Click the associated color box in the **Highlight Colors** dialog box.

   ⇒ The Color palette is displayed **(Figure 9.10)**.

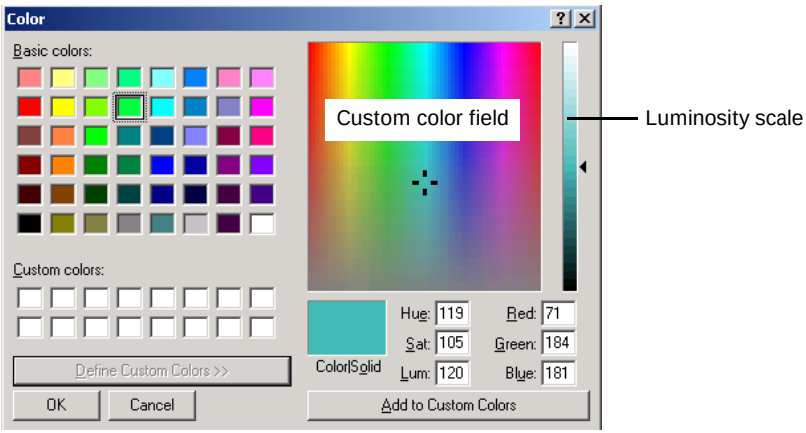


Figure 9.10
Color palette

b. To select a predefined color, click one of the basic colors.

c. To define a custom color, click **Define Custom Colors**, then use the click-and-drag method to move the cross hairs in the custom color field. Adjust the color brightness using the luminosity scale to the right. When finished, click **Add to Custom Colors** to apply the color.

   ⇒ The new color is applied to the selected item.

d. Click **OK** to close the Color palette.

Image Display Options

Intensity Display

You can view the probe cell intensity in the image data as a measured, average, or difference image.

1. Open the image data.
2. Select **View →Image** from the menu bar, then choose one of the three viewing options: **Measured**, **Difference**, or **Average**.

Measured Image View

The measured image view **(Figure 9.11)** displays the raw data from a scanned image.

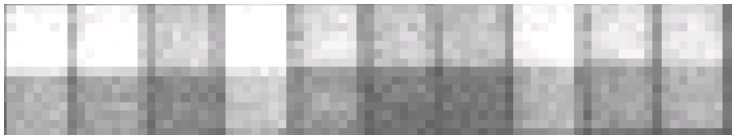


Figure 9.11
Measured image view

Cell Image View

The cell image view **(Figure 9.12)** displays the probe cell intensity values calculated by the Cell Analysis algorithm.

Figure 9.12
Cell image view

Difference Image View

The difference image view **(Figure 9.13)** displays the absolute difference between the measured and the cell intensity values for each pixel. The difference image of a probe cell with hybridized target typically displays high intensity at the perimeter where there is a large difference between the measured and the cell intensity value, but displays low intensity at the center due to small differences between the measured and the cell intensity values.

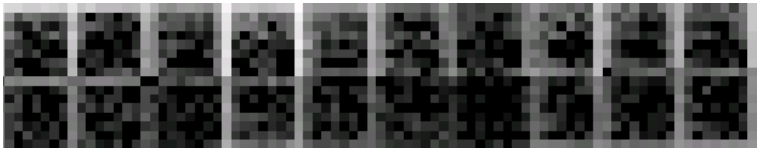


Figure 9.13
Difference image view

The difference image of a hybridized probe array is the reverse of the measured image **(Figure 9.11)** where the hybridized probe cells appear bright at the center and dark at the perimeter. The difference image helps assess the uniformity of the hybridization signal in the probe cell and whether the grid lines properly delineate the probe cells.

Viewing Probe Cell Data

You can click a probe cell (feature) in the image or cell intensity data to obtain more information about the feature and the associated gene.

To display the probe cell data for image or cell intensity data, do one of the following:

- Double-click the probe cell of interest.
- Click the probe cell of interest; right-click the image, and select **View Probe Cell Data** in the shortcut menu that appears.
- Click a probe cell of interest and select **View** →**Probe Cell Data** from the menu bar.

⇒ The Probe Cell Data dialog box appears **(Figure 9.14)**.

Click other probe cells in the image to update the displayed data.

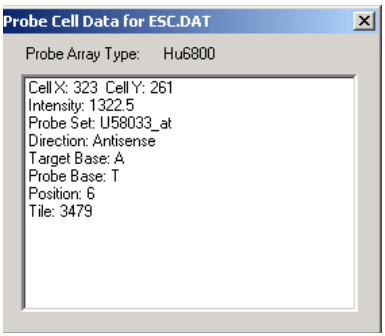


Figure 9.14
Probe cell data, GeneChip® Hu6800 probe array

Cell Coordinates	The probe cell x and y coordinates in cell units.
Intensity	The cell intensity calculated by the cell analysis algorithm.
Probe Set	The identifier of the probe set containing the probe cell.
Direction	The direction (sense or antisense) of the target (sample).
Target Base	The expected nucleotide at the position that corresponds to the substitution position of the probe.
Probe Base	The actual nucleotide located at the substitution position of the probe.
Position	The numbered position of the perfect match and mismatch probe pair within the probe set (for example, 1-20 for a probe set consisting of 20 probe pairs).
Tile	The identification number for the probe set.

Viewing Probe Set Information

Viewing Probe Array Tiling Information

You can view probe array tiling information for the current image or cell intensity data in the Image window.

1. Right-click the image or cell intensity data and click **View Probe Tiling** in the shortcut menu that appears. Alternatively, select **View →Probe Tiling** from the menu bar.

   ⇒ The **View Probe Tiling** dialog box appears **(Figure 9.15)**.

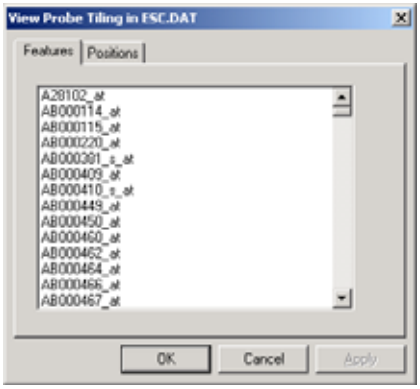


Figure 9.15
View Probe Tiling dialog box

The Features tab of the View Probe Tiling dialog box lists all probe sets tiled on the probe array for the current image or cell intensity data.

2. In the Features tab, press and hold the **Ctrl** key while you click the features of interest **(Figure 9.15)**. Click **Apply** when the selection is completed.

   ⇒ This highlights the probe sets in the image or cell intensity data that correspond to the selected features so that hybridization data may be quickly located **(Figure 9.16)**.

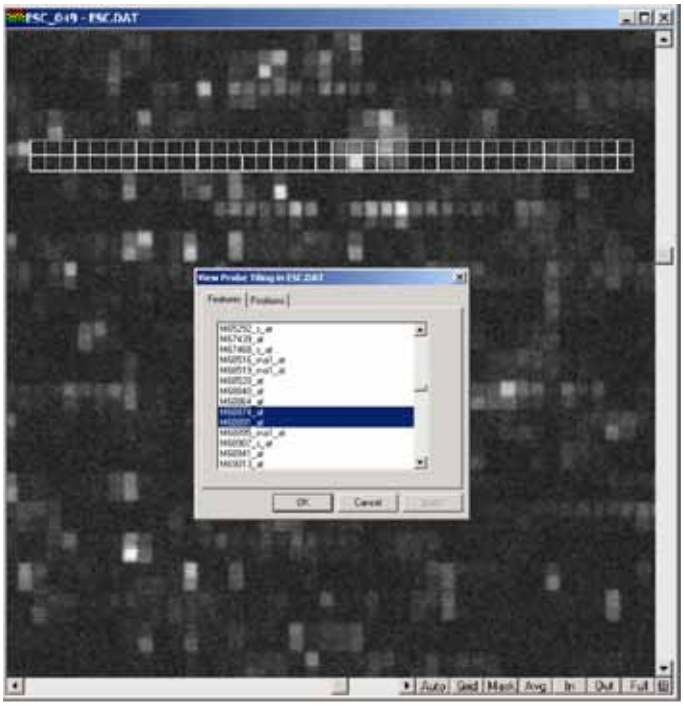


Figure 9.16
Highlighted probe cells in the image data

TIP

*Turn off the grid (press the **G** key) so that the highlighted probe cells can be easily seen. The highlighting color for the selected probe cells may be changed. See Specifying Highlight Colors on page 148.*

3. Click **OK** to close the View Probe Tiling dialog box.

Viewing Probe Cell Data for a Highlighted Probe Set(s)

1. In the image or cell intensity data, click a probe cell of interest.

   ⇒ This places the red cross hairs over the feature.

2. Right-click and select **View Probe Cell Data** from the shortcut menu that appears. Alternatively, select **View →Probe Cell Data** from the menu bar.

   ⇒ This displays the probe cell data **(Figure 9.17)**.

✔ Note *If you click another probe cell, GCOS automatically updates the probe Cell Data dialog box.*

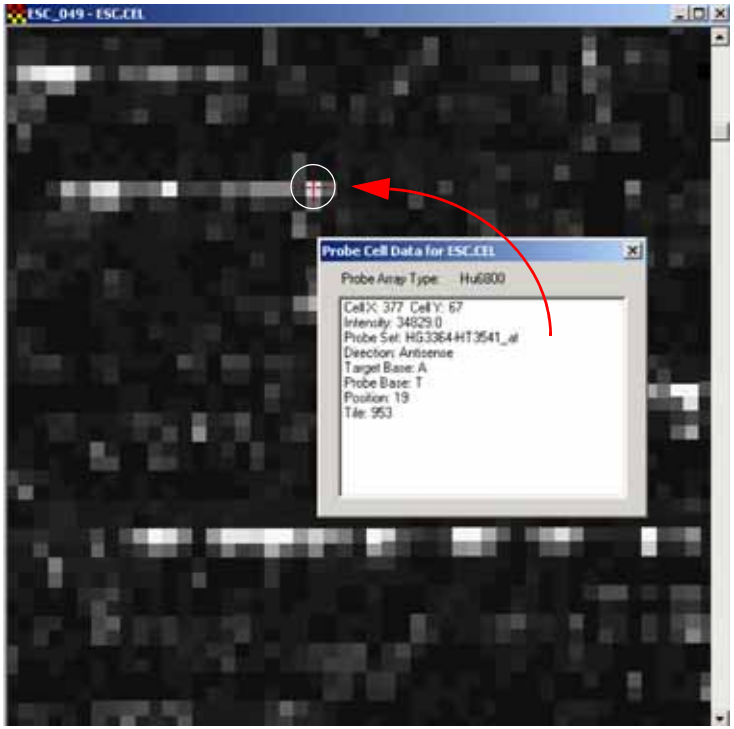


Figure 9.17
Probe cell data for a selected feature

3. To place the red cross hairs over the next feature of the highlighted probe set, click the image (to return focus to the Image window) and do one of the following:
   - Press the **N** key.
   - Right-click the image and select **View Next Highlight** in the shortcut menu that appears.
   - Select **View** →**Next Highlight** from the menu bar.

   If it is open, the Probe Cell Data dialog box is automatically updated with the new probe cell data.

4. To return the cross hairs to a previous feature, do one of the following:
   - Press the **P** key.
   - Right-click the image and select **View Previous Highlight** in the shortcut menu that appears.
   - Select **View** →**Previous Highlight** from the menu bar.
5. To remove all highlights, do one of the following:
   - Press the **E** key.
   - Right-click the image and select **Clear Highlights** from the shortcut menu that appears.
   - Select **View** →**Clear Highlights** from the menu bar.

✔ Note

Highlights are automatically cleared when you close the image or cell intensity data.

Highlighting Tiles on the Probe Array

You can highlight:

- all tiles or a particular tile on the probe array (by tile number)
- all positions or a particular position of all tiles or a selected tile

1. Right-click the image or cell intensity data and click **View Probe Tiling** in the shortcut menu that appears. Alternatively, select **View** →**Probe Tiling** from the menu bar.

   ⇒ The View Probe Tiling dialog box appears **(Figure 9.18)**.
2. Click the Positions tab, select a **Position** and **Tile**, then click **Apply.**

   ⇒ This highlights the selection in the image.

   Figure 9.18 shows two examples: all positions of tile number 3000 highlighted and only position 15 of tile 3000 highlighted.

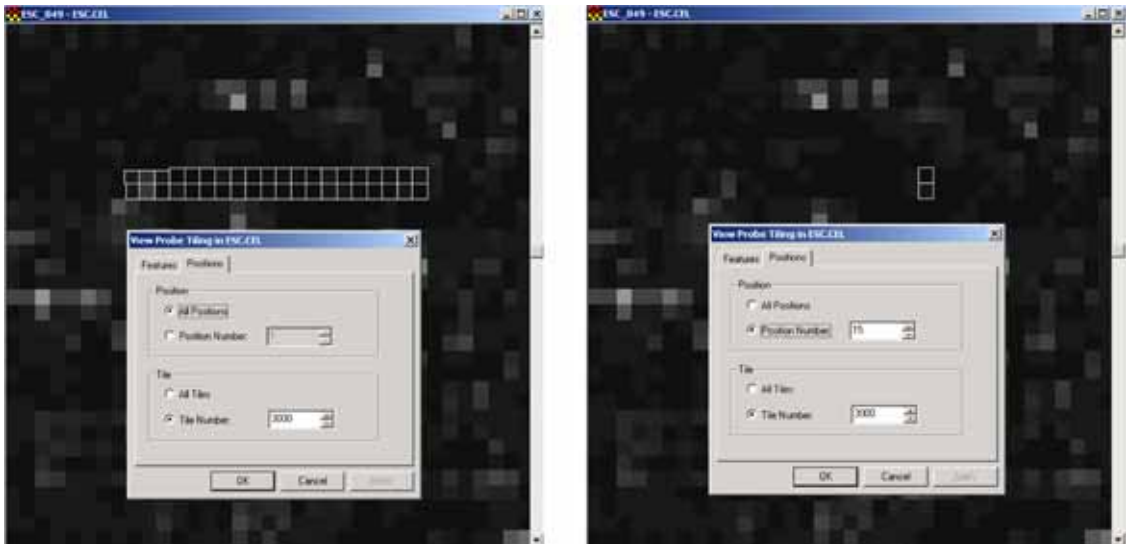


Figure 9.18
Cell intensity data, all positions of tile 3000 highlighted (left), position 15 of tile 3000 highlighted (right)

Bookmarks

A bookmark denotes a specific area of the image or cell intensity data at a particular magnification level. Bookmarks make it easy to quickly view the hybridization data at a specific location in different images.

When you select a bookmark, the Image window automatically displays the image location at the magnification specified by the bookmark.

Creating a Bookmark

1. Use the task bar zoom commands and scroll bars in the Image window to locate the image area of interest at the desired magnification level.
2. Right-click the image and select **View Bookmarks →Add Bookmark** from the shortcut menu that appears. Alternatively, select **View → Bookmarks →Add Bookmark** from the menu bar.

   ⇒ The Add Bookmark dialog box appears **(Figure 9.19)**.

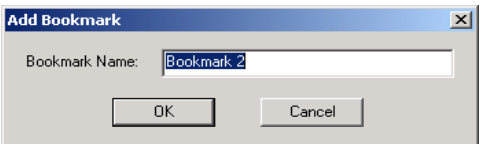


Figure 9.19
Add Bookmark dialog box

3. Enter a name in the **Bookmark Name** box or use the default name **Bookmark 1, 2,3...**.
4. Click **OK** to create the bookmark.

Viewing Bookmarks

To view bookmarks, do either of the following:

- Right-click the image and select **View Bookmarks** →**Edit Bookmarks** from the shortcut menu that appears. Alternatively, select **View** → **Bookmarks** →**Edit Bookmarks** from the menu bar.

  ⇒ The Edit Bookmarks dialog box displays a list of all the bookmarks **(Figure 9.20)**.
- Select **View** →**Bookmarks** from the menu bar. (A maximum of 20 bookmark names are displayed here.)

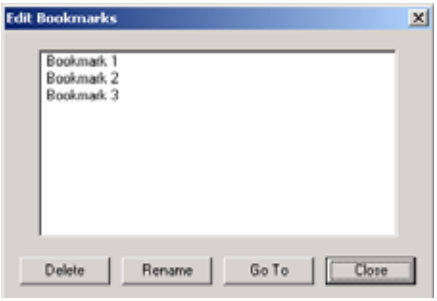


Figure 9.20
Edit Bookmarks dialog box

Commands in the Edit Bookmarks Dialog Box

Highlight the bookmark name in the Edit Bookmarks dialog box, then click the command of interest.

Delete	Permanently removes the selected bookmark.
Rename	Allows the selected bookmark to be renamed.
Go To	Displays the selected bookmark in the image.
Close	Closes the Edit Bookmarks dialog box.

Outliers

Outliers are probe cells that are obscured or non-uniform in intensity (for example, probe cells with bright or dark streaks). The Outlier Detection algorithm automatically determines whether a probe cell is an outlier.

You can exclude or *mask* outliers from a probe array in an expression analysis. The Statistical Expression algorithm (generates the probe analysis data from the cell intensity data) ignores the probe cell data from masked outliers.

Viewing Outliers

1. Open the image or cell intensity data of interest.
2. Right-click in the Image window and select **View →Cells Masked** from the shortcut menu. Alternatively, select **View →Cells →Outliers** from the menu bar.

   ⇒ This highlights outlier probe cells with diagonal lines .

✔ Note

The color that highlights outliers may be changed. See *Specifying Highlight Colors on page 148.*

Masking Outliers

1. Open the image or cell intensity data of interest.
2. Select **Edit →Mask All Outliers** from the menu bar.
   ⇒ All outliers identified by the Outlier Detection algorithm are masked.
3. To mask user-specified probe cells:
   a. Use the click-and-drag method to select a probe cell or area of the image to be masked.
   b. Select **Edit →Mask Cells** from the menu bar.

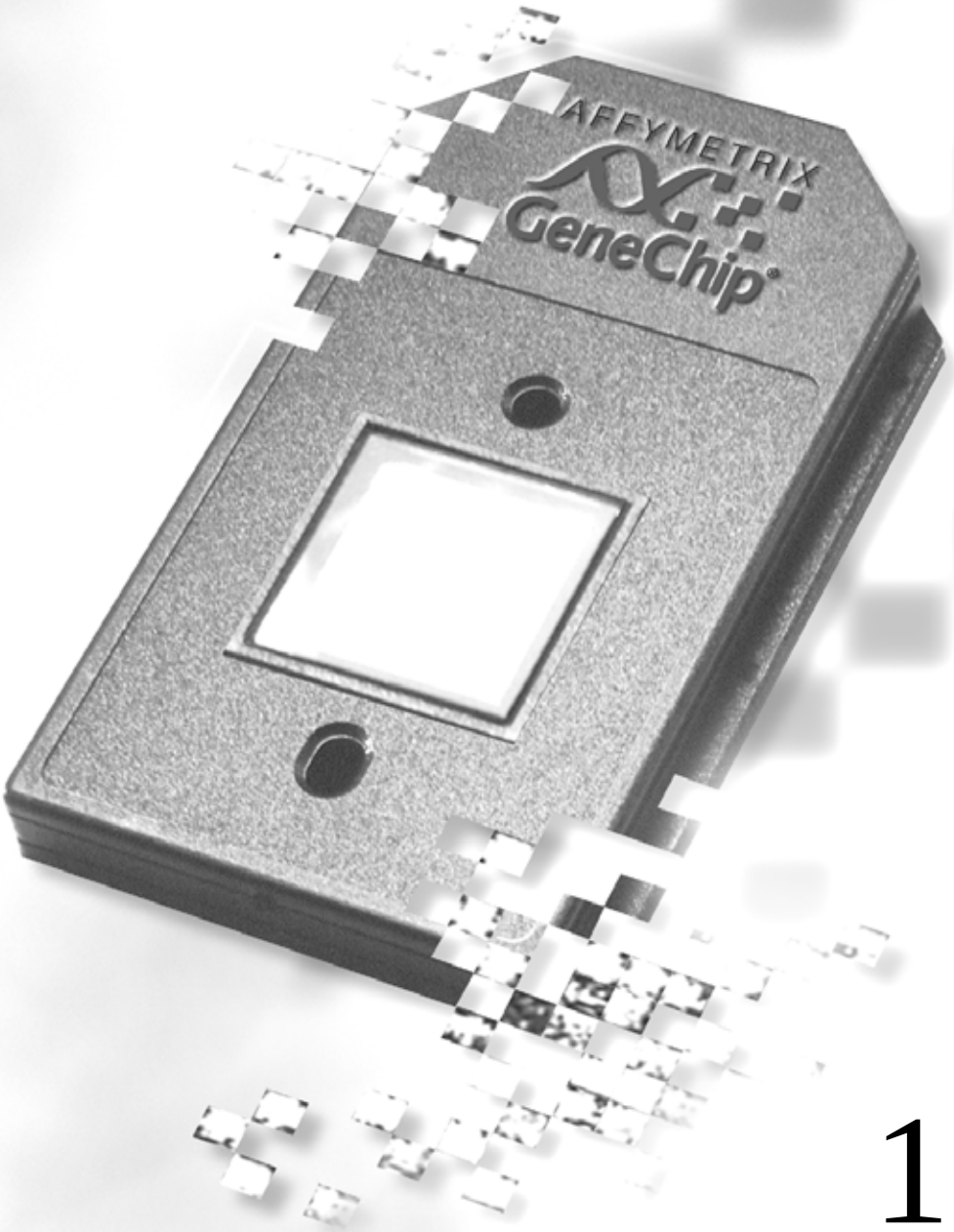
TIP *Turn off the grid (press the **G** key) to easily view the masked probe cells highlighted with diagonal lines.*

4. To view masked probe cells, right-click in the Image window and select **View Cells →Masked** from the shortcut menu that appears.

Unmasking Outliers

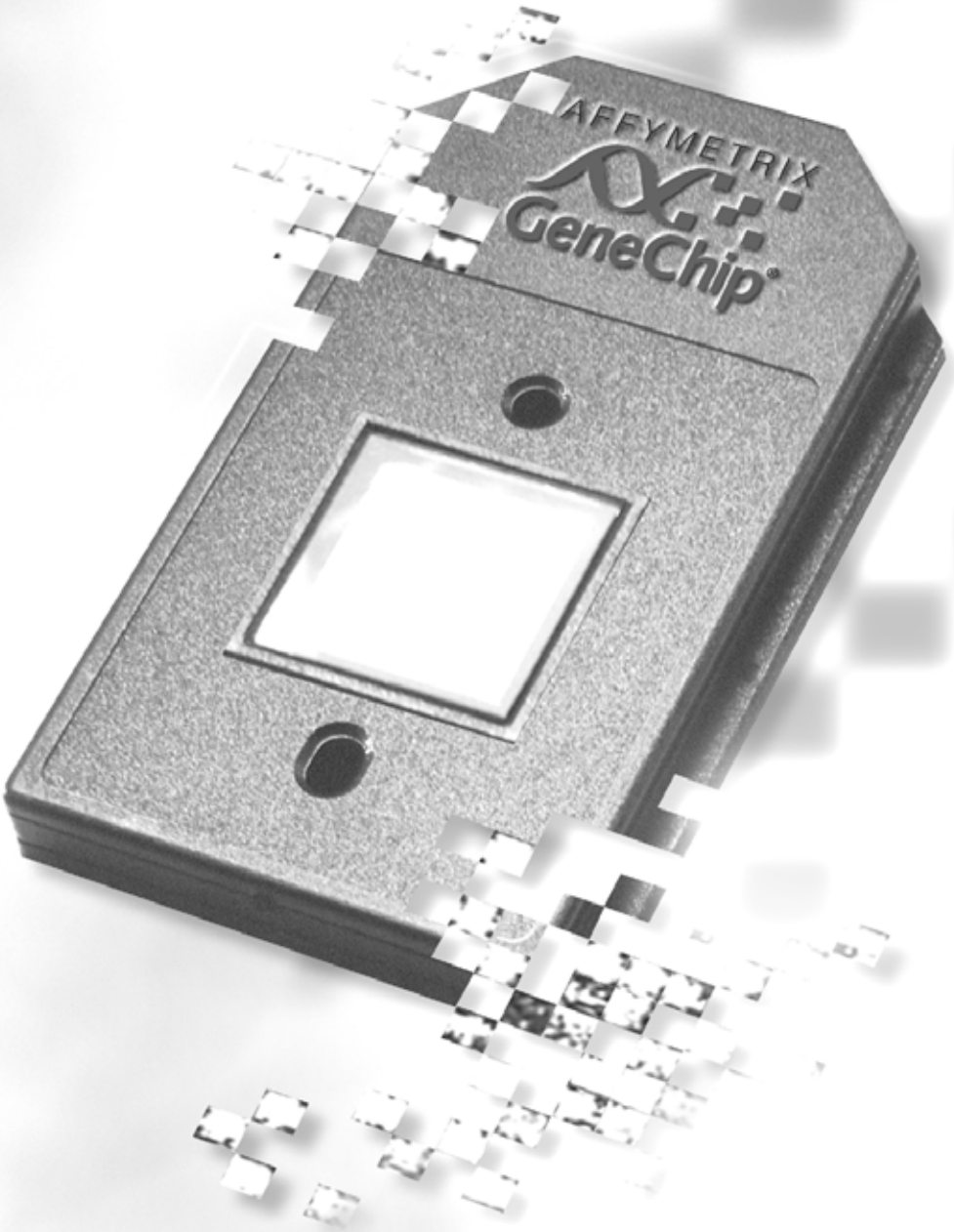
1. Select **Edit →Unmask All Cells** from the menu bar.
   ⇒ This removes all masking.
2. To unmask only a selected probe cell or area:
   a. Use the click-and-drag method to select a probe cell or area of interest.
   b. Select **Edit →Unmask Cells** from the menu bar.

Note *If you manually adjust the grid or change the probe array type in the experiment, the mask or outlier data will be lost when new cell intensity data are generated.*



10

Chapter 10



Gene Expression Analysis

The Expression Analysis algorithm analyzes cell intensity data from GeneChip® expression arrays **(Figure 10.1)**.

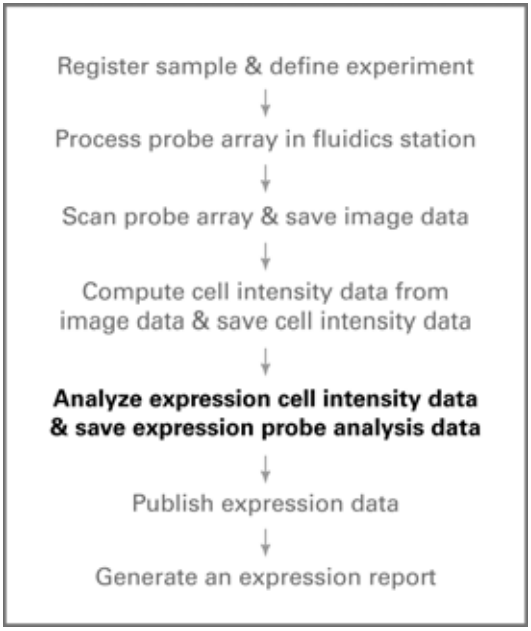


Figure 10.1
Assay & analysis flow chart

This chapter explains how to:

- perform single-array or comparison array expression analysis
- view the expression probe analysis data in graphical or tabular format in the Expression Analysis window (EAW)
- generate a report

Overview of Single-Array & Comparison Array Expression Analysis

Single-Array Expression Analysis

A single-array expression analysis examines the cell intensity data from one experiment (GeneChip® probe array). For each transcript represented on the probe array, the expression algorithm computes the:

- Detection call (present, absent, or marginal (unable to call the transcript present or absent), or no call)
- Detection *p*-value
- Signal (background-subtracted and adjusted for noise)
- Stat Pairs
- Stat Pairs Used

GCOS saves the probe analysis or *chip* data.

To run a single-array analysis **(Figure 10.2)**:

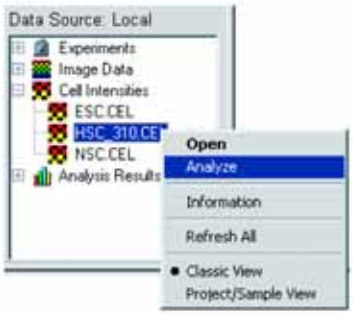
- confirm the defaults or specify new values in the Expression Analysis Settings dialog box
- in the data tree, select the cell intensity data for the analysis
- analyze the cell intensity data and view the probe analysis data in the Expression Analysis window (EAW)

Single-Array Expression Analysis

1. **Expression Analysis Settings**

   In the Settings shortcut bar, click the shortcut button to open the Expression Analysis Settings dialog box. Confirm or specify new analysis settings. Do not choose Use Baseline Comparison File option.

2. **Select & Analyze Cell Intensity Data** In the data tree, right-click the cell intensity data and select **Analyze** from the shortcut menu.



3. **View Probe Analysis Data** Expression Analysis window (EAW) displays single-array probe analysis data.

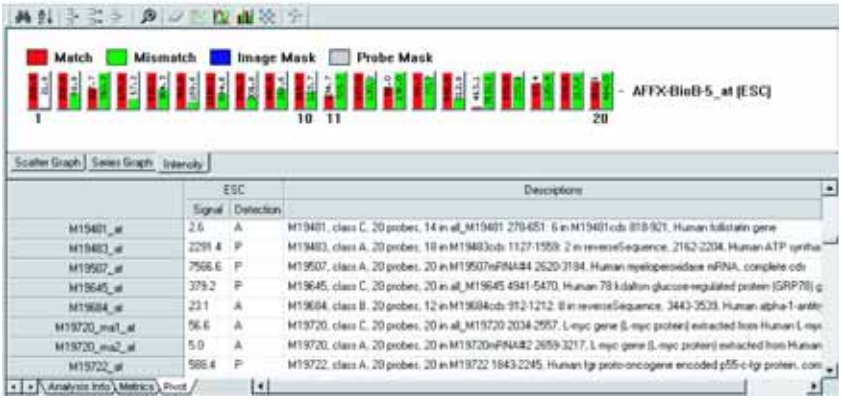


Figure 10.2
Single-array expression analysis

Comparison Expression Analysis of an Experiment & Baseline

A comparison analysis compares the cell intensities of the same probe set on two different probe arrays (an experiment and baseline of the same probe array type) to determine the relative change in the expression level of a transcript.

For each transcript represented on the probe array, the expression algorithm computes the:

- Change call (increase, marginal increase, no change, marginal decrease, decrease) or no call
- Change Detection *p*-value
- Signal Log Ratio (background-subtracted and adjusted for noise)
- Signal Log Ratio Low
- Signal Log Ratio high
- Stat Common Pairs

GCOS saves the probe analysis data.

To run a comparison analysis **(Figure 10.3)**:

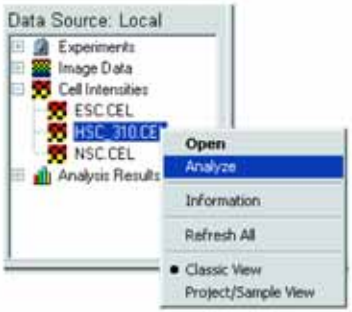
- select the baseline probe analysis data
- confirm the default expression analysis settings or specify new values
- select the experiment cell intensity data from the data tree
- analyze the experiment cell intensity data and view the comparison probe analysis data in the EAW

Comparison Array Expression Analysis Compares an Experiment & Baseline

1. **Expression Analysis Settings**

   In the Settings shortcut bar, click the shortcut button to open the Expression Analysis Settings dialog box. Confirm or specify new analysis settings. Choose the Use Baseline Comparison Data option. Click **Browse** to select the baseline probe analysis data.

2. **Select & Analyze Cell Intensity Data**
   In the data tree, right-click the cell intensity data and select **Analyze** from the shortcut menu.



3. **View Probe Analysis Data**
   Expression Analysis window (EAW) displays comparison analysis output.

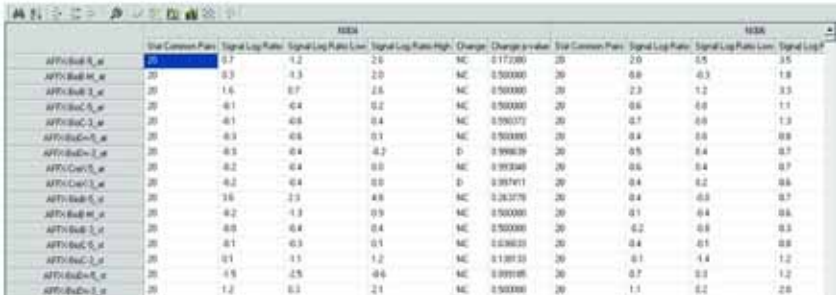


Figure 10.3
Comparison expression analysis of an experiment and baseline

Expression Analysis Settings

The Statistical Expression algorithm relies on the expression analysis settings to derive biologically meaningful results from the hybridization intensity data. The Expression Analysis Settings dialog box **(Figure 10.4)** displays the:

- the type of expression probe array for the analysis
- a scaling or normalization factor (for more information, see Appendix E, *Expression Algorithm, Expression Analysis Metrics & Settings*, on page 515)
- probe mask definition (for more information, see Appendix E on page 515)
- the baseline data selected for a comparison analysis
- the user-modifiable parameters of the expression analysis algorithm (for more information, see Appendix E on page 515)

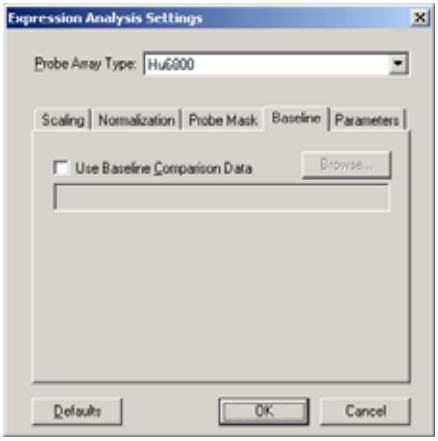


Figure 10.4
Expression Analysis Settings dialog box, Baseline tab

Note

Before running a single-array or comparison analysis, confirm the defaults or specify new values for the expression analysis settings.

1. To open the Expression Analysis Settings dialog box **(Figure 10.4)**, click **Expression** in the Settings shortcut bar.
2. Click a tab to view each type of setting. See *Expression Analysis Settings* on page 532 for more information about the settings and how to change them.

   The expression analysis settings are saved on a per user basis. Changes made by one user (identified by the logon name) do not affect the settings of other users.

TIP

To compare the probe analysis data of several experiments, scale the experiments to the same target intensity using the ***All Probe Sets*** *or* ***Selected Probe Sets*** *scaling option. See Scaling Tab on page 533.*

Single-Array Expression Analysis

A single-array expression analysis analyzes the cell intensity data of a GeneChip® expression probe array. For each transcript represented on the probe array, the algorithm computes a Detection call, Detection *p*-value, and Signal.

Note

An expression analysis run from the data tree, menu bar, toolbar, or workflow monitor uses the expression analysis settings in the Expression Analysis Settings dialog box. The userset specified during experiment setup is not applied. For more information on usersets, see Userset Tab on page 335.

1. Open the Expression Analysis Settings dialog box **(Figure 10.4)**, and:
   - select the probe array type for the baseline from the **Probe Array Type** drop-down list
   - click the Baseline tab and confirm that the **Use Baseline Comparison File** option is *not* chosen
   - confirm or change the other expression analysis settings as desired
2. To analyze cell intensity data, do one of the following:
   - in the data tree, right-click the cell intensity data name (or image data name), then click **Analyze** in the shortcut menu that appears

- select **File** →**Analyze** from the menu bar and choose the cell intensity data that you want to analyze from the Analyze dialog box that appears.
- if the cell intensity data or image data is open, click the **Analyze** . Alternatively, select **Run** →**Analysis** from the menu bar.

⇒ The Save Results As dialog box appears (if chosen as a default option, see *Analysis Settings* on page 263) and displays the probe analysis data default name (same as the experiment name specified during experiment setup) **(Figure 10.5)**.

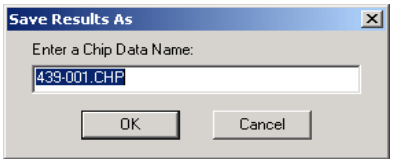


Figure 10.5
Save Results As dialog box

3. If an analysis was previously run and you want to save the current results without overwriting the previous results, enter a new name in the Save Results As dialog box.
4. Click **OK**.

   ⇒ This closes the Save As dialog box and runs the single-array analysis.

Note

The Expression Analysis Settings dialog box appears next if chosen as a default option. For more information on this default setting, see Analysis Settings on page 263.

When the analysis is finished, the Expression Analysis window (EAW) displays the probe analysis data **(Figure 10.6)**.

The status log **(Figure 10.6)** displays:

- the name of the probe analysis data
- the location of the probe analysis data
- a message indicating when the analysis is completed

If the status log is not displayed, click the **Status Log** button or select **View** →**Status Bar** from the menu bar.

✔ Note

If the EAW is already open, the results are added to the open window and it may be necessary to use the scrollbars at the bottom and right side of the EAW to see the newly added results.

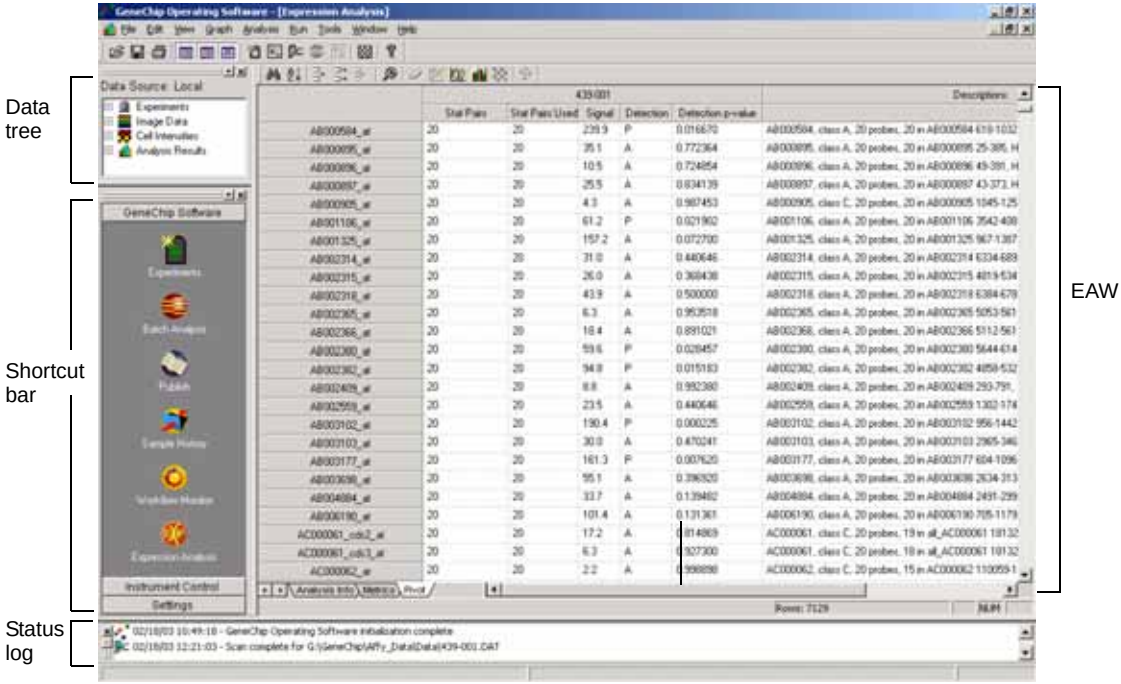


Figure 10.6
Expression Analysis Window (EAW), pivot tab, single-array analysis data

Comparison Expression Analysis

A comparison expression analysis compares the cell intensity data of an experiment to a baseline GeneChip® expression probe array (of the same probe array type). The comparison analysis identifies the relative change in the expression level of each transcript represented on the probe array.

For each transcript represented on the array, the Statistical Expression algorithm computes a Change call, Change p-value, Signal Log Ratio, Signal Log Ratio low, and Signal Log Ratio high.

Note

A single-array analysis of the baseline must be run prior to running a comparison analysis of the experiment and baseline.

Selecting the Baseline

1. Open the Expression Analysis Settings dialog box **(Figure 10.7)** (click **Expression** in the Settings shortcut bar).
2. Select the probe array type for the baseline from the **Probe Array Type** drop-down list.

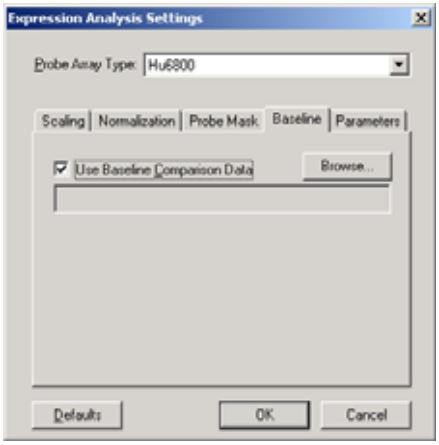


Figure 10.7
Expression Analysis Settings dialog box

3. Click the Baseline tab and choose the **Use Baseline Comparison File** option.

4. Click **Browse**.

   ⇒ The Open dialog box appears **(Figure 10.8)**.

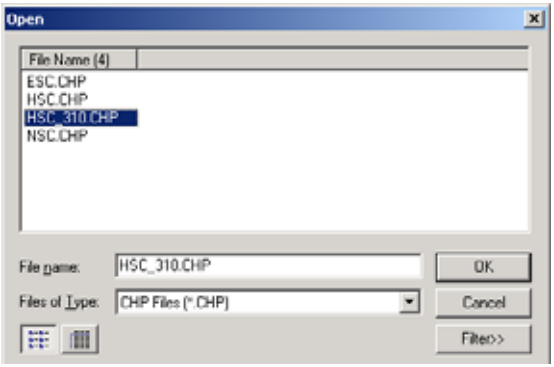


Figure 10.8
Open dialog box

5. Select the baseline data and click OK.

Running the Comparison Analysis

1. To run the comparison analysis of an experiment and baseline, do one of the following:
   - in the data tree, right-click the experiment cell intensity data or image, and click **Analyze** in the shortcut menu that appears
   - select **File** →**Analysis** from the menu bar and choose the experiment cell intensity data from the Analyze dialog box that appears
   - if the cell intensity data or image is open, click the **Analyze** button . Alternatively, select **Run** →**Analysis** from the menu bar.

   ⇒ The Save Results As dialog box appears (if chosen as a default option, see *Analysis Settings* on page 263) and displays the default name for the probe analysis data (same as the experiment name specified during experiment setup) **(Figure 10.9)**.

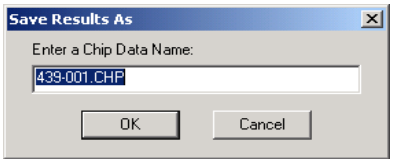


Figure 10.9
Save Results As dialog box

2. Enter a new name in the **Save Results As** dialog box if an analysis was previously run and you want to save the current results without overwriting the previous results. Click **OK**.

   ⇒ The **Expression Analysis Settings** dialog box appears next if chosen as a default option **(Figure 10.10)**. For more information on this default setting, see *Analysis Settings* on page 263.

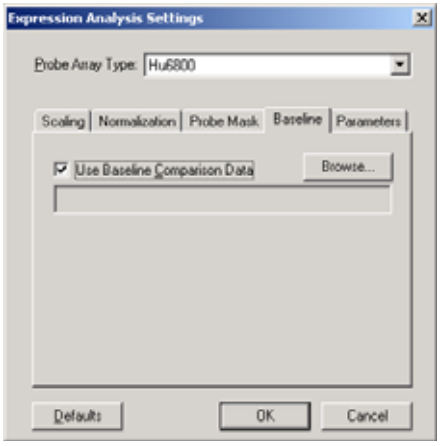


Figure 10.10
Expression Analysis Settings dialog box, baseline tab

3. If the Expression Analysis Settings dialog box appears, review and confirm the settings for the probe array type and baseline data:

   a. In the Baseline tab, select the probe array type for the baseline from the **Probe Array Type** drop-down list **(Figure 10.10)**.

b. Choose the **Use Baseline Comparison Data** option, then click **Browse**

⇒ The Open dialog box appears **(Figure 10.11)**.

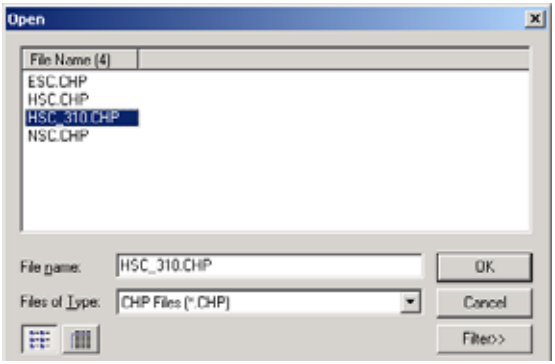


Figure 10.11
Open dialog box

c. Double-click the baseline data. Alternatively, click the baseline data, then click **Open** or **OK**.

⇒ The Expression Analysis Settings dialog box displays the selected baseline data (.chp) **(Figure 10.12)**.

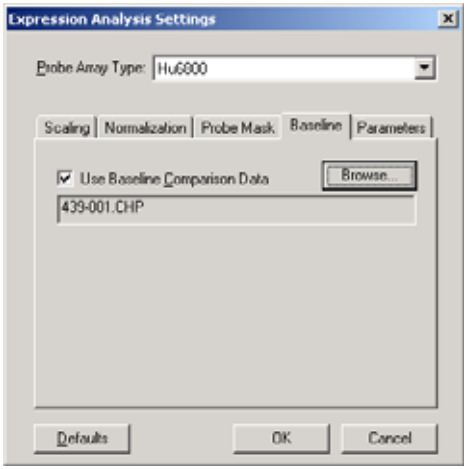


Figure 10.12
Expression Analysis Settings, baseline tab displays the baseline data for the comparison analysis

4. In the Expression Analysis Settings dialog box **(Figure 10.12)**, click the normalization tab and specify a normalization option for the experimental data.

✔ Note

You need only specify a normalization option for the experiment, not the baseline. You may use the scaling option instead of the normalization option. If you use the scaling option, scale both the experiment and the baseline. For more details on normalization and scaling, see Appendix E, *Expression Algorithm, Expression Analysis Metrics & Settings, on page 515.*

5. Confirm or specify other probe array analysis parameters as desired. For more details on the parameters, see Appendix E, *Expression Algorithm, Expression Analysis Metrics & Settings*, on page 515.

6. Click **OK**.

   ⇒ The Expression Analysis Settings dialog box closes and the comparison analysis runs.

   When the analysis is finished, the Expression Analysis window (EAW) displays the probe analysis data **(Figure 10.13)**.

The status log **(Figure 10.13)** displays:

- the name of the probe analysis data
- the location of the probe analysis data
- a message indicating when the analysis is completed

If the status log is not displayed, click the **Status Log** button or select **View →Status Bar** from the menu bar.

Note

If the EAW is already open, the results are added to the open window and it may be necessary to use the scrollbars at the bottom and right side of the EAW to see the newly added results.

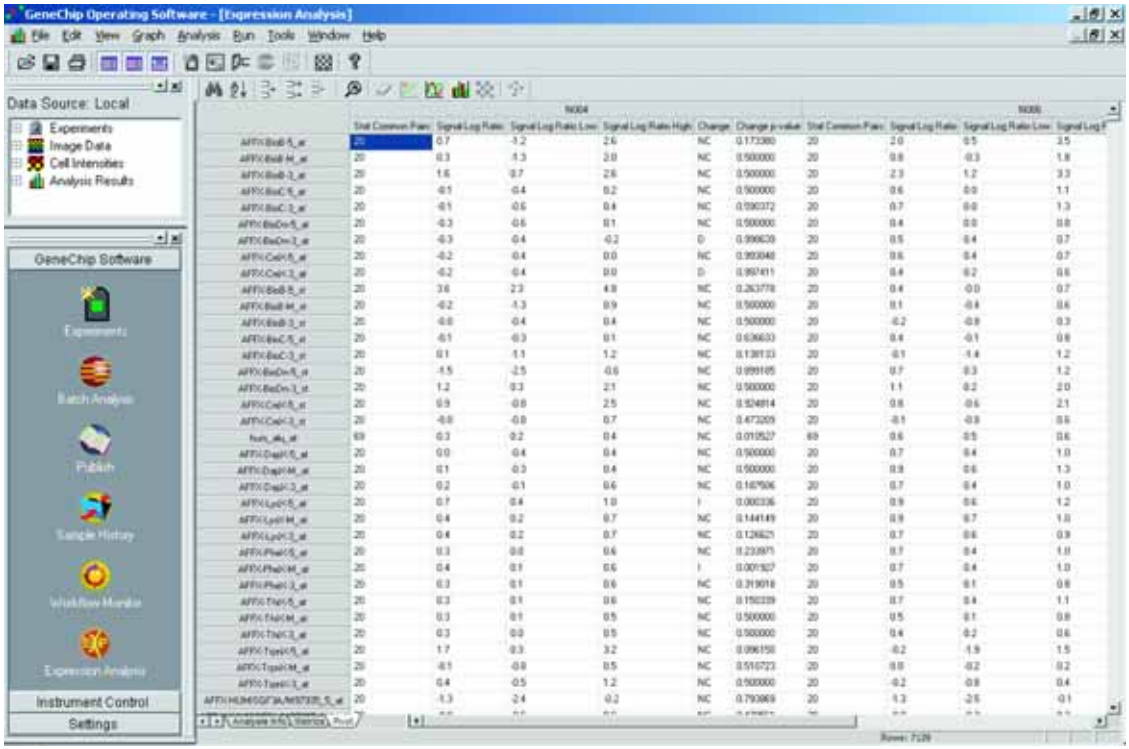


Figure 10.13
Expression Analysis Window (EAW), comparison probe analysis data, pivot tab

Expression Analysis Tabular Data

The Expression Analysis window (EAW) has three tables that display the tabular and graphic analysis output for gene expression assays:

- Analysis information table
- Metrics table
- Pivot table

The EAW opens after you complete an expression analysis or when you open expression probe analysis data.

You can open more than one set of probe analysis data in the EAW. Use the EAW scroll bars to view all of the results. A second toolbar and additional menu bar commands are available in the EAW (see Appendix I, *Toolbars, Hot Keys, & Windowpanes*, on page 613).

Analysis Information Table

The analysis information table **(Figure 10.14)** displays experiment and sample information entered during experiment setup, values for user-modifiable expression analysis algorithm settings, and other information. Each column in the analysis information table represents one analysis (probe array).

	Hu6800	sj0305060a
Probe Array Lot:	90081	
Operator Name:	Affy User	
Sample Type:	Total RNA	
Sample Description:		
Project:	Human	
Comments:	(null)	
Reagents:		
Reagent Lot:		
Algorithm Type:	ExpressionStat	ExpressionStat
Algorithm Version:	5.0	5.0
Corner+	Avg:182, Count:32	Avg:85, Count:32
Corner-	Avg:11483, Count:32	Avg:1861, Count:32
Background	Avg:636.30,Stdev:14.36,Max:674.9,Min:603.8	Avg:7261.17,Stdev:356.48,Max:8089.4,Min:6246.2
Noise	Avg:16.43,Stdev:0.67,Max:18.4,Min:14.7	Avg:189.16,Stdev:48.86,Max:382.8,Min:108.8
RawQ	23.54	7.80
BF	D:\GeneChipDB\Data\Hu6800.CHP	D:\GeneChipDB\Data\sj0305060a_baseline.CHP
Alpha1	0.04	0.04
Alpha2	0.06	0.06
Tau	0.015	0.015
Gamma1H	0.0025	0.0025
Gamma1L	0.0025	0.0025
Gamma2H	0.0030	0.0030
Gamma2L	0.0030	0.0030
Perturbation	1.08	1.08
NF	1.000000	1.000000
SF	1.000000	24.513575

Analysis Info / Metrics / Pivot

Figure 10.14
Expression Analysis window, analysis information table

Exporting Analysis Information

You can export the data in the analysis information table to a tab delimited text file (.txt) or Microsoft® Excel file (.xls).

1. If it is not already open, open the EAW (in the data tree, double-click the probe analysis data that you want to export).
2. Click the Analysis Information tab.
3. To export a user-selected portion of the data table, highlight the cells of interest.
4. Click the **Save** button . Alternatively, select **File** →**Save As** from the menu bar.

   ⇒ The Save As dialog box appears **(Figure 10.15)**.

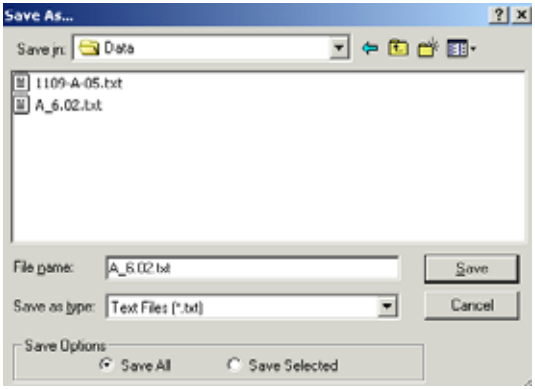


Figure 10.15
Save As dialog box

5. Choose a **Save Option**:
   - **Save All** exports all of the analysis information table data to the text file.
   - **Save Selected** exports only the user-selected portion of the analysis information table to the text file.
6. Enter a name for the text file and confirm or select another directory for the text file.

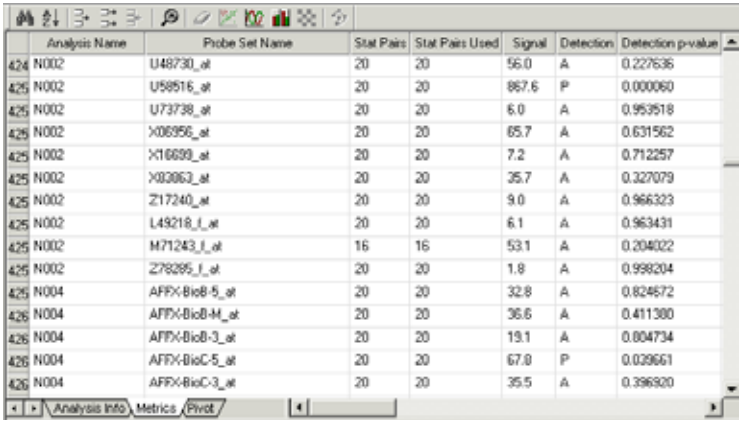
7. Click **Save** to export the experiment information table or selected experiment data.

Metrics Table

The metrics table **(Figure 10.16)** displays data for each probe set in the selected probe analysis data (**Table 10.1**). See *Expression Analysis Metrics*, on page 524 for definitions of the Statistical expression metrics.

Table 10.1
Statistical expression algorithm metrics

Single-Array Analysis	Comparison Analysis
Stat Pairs	Stat Common pairs
Stat Pairs Used	Signal Log Ratio
Signal	Signal Log Ratio low
Detection	Signal Log Ratio high
Detection *p*-value	Change
	Change p-value



	Analysis Name	Probe Set Name	Stat Pairs	Stat Pairs Used	Signal	Detection	Detection p-value
424	N002	U48730_at	20	20	56.0	A	0.227636
425	N002	U58516_at	20	20	867.6	P	0.000060
425	N002	U73738_at	20	20	6.0	A	0.953518
425	N002	X06956_at	20	20	65.7	A	0.631562
425	N002	X16699_at	20	20	7.2	A	0.712257
425	N002	X83863_at	20	20	35.7	A	0.327079
425	N002	Z17240_at	20	20	9.0	A	0.966323
425	N002	L49218_f_at	20	20	6.1	A	0.963431
425	N002	M71243_f_at	16	16	53.1	A	0.204022
425	N002	Z78285_f_at	20	20	1.8	A	0.998204
426	N004	AFFX-BioB-5_at	20	20	32.8	A	0.824672
426	N004	AFFX-BioB-M_at	20	20	36.6	A	0.411380
426	N004	AFFX-BioB-3_at	20	20	19.1	A	0.804734
426	N004	AFFX-BioC-5_at	20	20	67.8	P	0.029661
426	N004	AFFX-BioC-3_at	20	20	35.5	A	0.396920

Analysis Info | Metrics | Pivot

Figure 10.16
Expression Analysis window, metrics tab, single-array probe analysis data for experiments N002 and N004

Exporting Metrics Data

You can export metrics data and the associated analysis information as a text file (.txt) or Microsoft® Excel file (.xls).

Setting Metrics Export Preferences

1. Click the **Analysis Options** button .
   ⇒ The Analysis Options dialog box appears **(Figure 10.17)**.
2. Click the metrics tab to view export preferences.

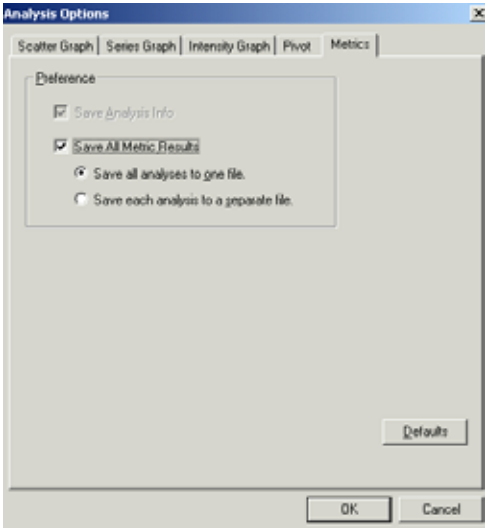


Figure 10.17
Analysis Options, Metrics tab

3. Choose the **Save Analysis Info** option to include the analysis information with the metrics data for export.
4. Choose the **Save All Metric Results** option to view the save preferences:
   - The **Save all analyses to one file** option exports the analysis information and metrics data for all analyses in the EAW to one text file (.txt).

- The **Save each analysis to a separate file** option exports the analysis information and metrics data for each analysis in the EAW to a separate text file.

✔ Note

The save preferences set in the metrics tab do not affect the save function in the Pivot or Analysis Information tab in the EAW.

5. Click **OK** to close the Analysis Options dialog box.

Exporting Metrics Data

1. If it is not already open, open the EAW (in the data tree, double-click the probe analysis data of interest), then click the Metrics tab.
2. To export:
   - All of the metrics table, click the **Save** button. Alternatively, select **File** →**Save As** from the menu bar.
   - A selected portion of the metrics table, highlight the rows of interest, then click the **Save** button or select **File** →**Save As** from the menu bar.

✔ Note

*Do not choose the **Save All Metric Results** option* **(Figure 10.17)** *if you want to save selected results.*

⇒ The Save As dialog box appears **(Figure 10.18)**.

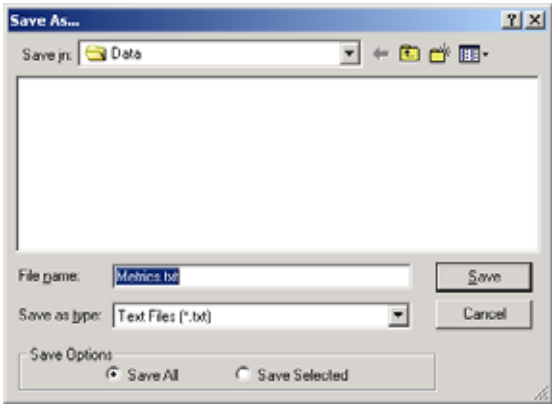


Figure 10.18
Save As dialog box

3. Enter a name for the text file and confirm or select another directory for the text file.
4. Click **Save** to export the metrics data according to the preferences set in the Analysis Options (Metrics tab) **(Figure 10.17)**.

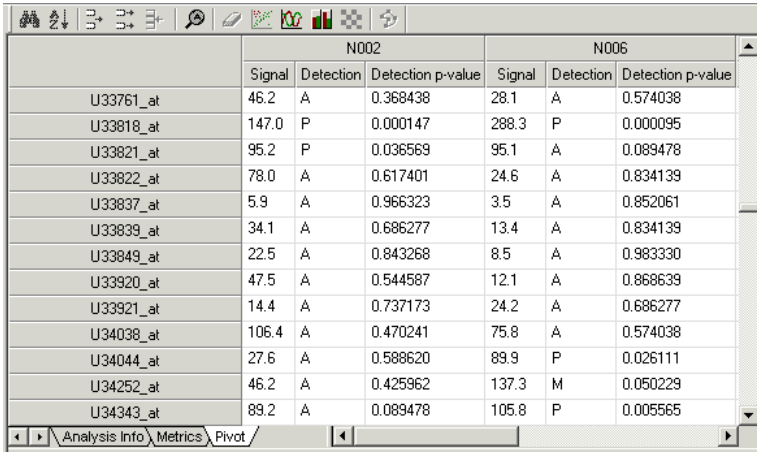
Pivot Table

To view the pivot table:

- open probe analysis data (double-click the data in the data tree)
- click the Pivot tab in the EAW

Each row in the pivot table **(Figure 10.19)** begins with a probe set name and each set of columns displays the results from one probe analysis. The pivot table displays probe analysis data and descriptions for each transcript represented on the probe array.

If several sets of probe analysis data from the same probe array type are open in the EAW, each row of the pivot table displays the data for one probe set from the different experiments. The pivot table is organized so that probe analysis data from different experiments may be easily compared.



	N002			N006		
	Signal	Detection	Detection p-value	Signal	Detection	Detection p-value
U33761_at	46.2	A	0.368438	28.1	A	0.574038
U33818_at	147.0	P	0.000147	288.3	P	0.000095
U33821_at	95.2	P	0.036569	95.1	A	0.089478
U33822_at	78.0	A	0.617401	24.6	A	0.834139
U33837_at	5.9	A	0.966323	3.5	A	0.852061
U33839_at	34.1	A	0.686277	13.4	A	0.834139
U33849_at	22.5	A	0.843268	8.5	A	0.983330
U33920_at	47.5	A	0.544587	12.1	A	0.868639
U33921_at	14.4	A	0.737173	24.2	A	0.686277
U34038_at	106.4	A	0.470241	75.8	A	0.574038
U34044_at	27.6	A	0.588620	89.9	P	0.026111
U34252_at	46.2	A	0.425962	137.3	M	0.050229
U34343_at	89.2	A	0.089478	105.8	P	0.005565

Figure 10.19
Expression Analysis window (EAW), pivot table, single-array probe analysis data for experiments N002 and N006

Pivot Table Display Options

Single-array or comparison analysis output

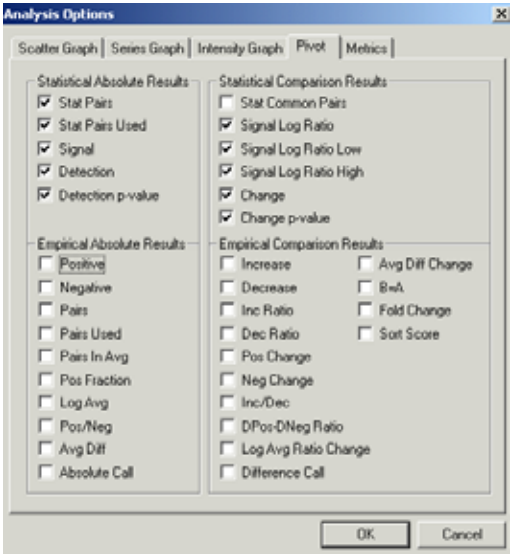
You can select the types of single-array or comparison analysis output (columns) displayed in the pivot table.

1. Click the **Analysis Options** button. Alternatively, select **Analysis** → **Options** from the menu bar.

   ⇒ The Analysis Options dialog box appears **(Figure 10.20)**.

2. Click the Pivot tab.

   The pivot table includes column headers for the items selected in the Analysis Options dialog box. You can also view these items by selecting **View** →**Pivot Data** from the menu bar.



Select the output for display in the Pivot table.

Figure 10.20
Analysis Options dialog box, Pivot tab

Note

The pivot table can also display probe analysis data for experiments analyzed using the Empirical expression analysis algorithm (in Microarray Suite versions lower than 5.0).

3. Click an unchecked item to add it to the pivot table or click a checked item to remove it from the table.

4. To reorder an experiment result column in the pivot table, use the drag-and-drop method to move the column header in the table.

Probe Set Descriptions

To view probe set descriptions, do either of the following:

- scroll to the Descriptions (far right column) in the pivot table
- double-click the row of interest in the pivot table

  ⇒ The Description dialog box is displayed **(Figure 10.21)**.

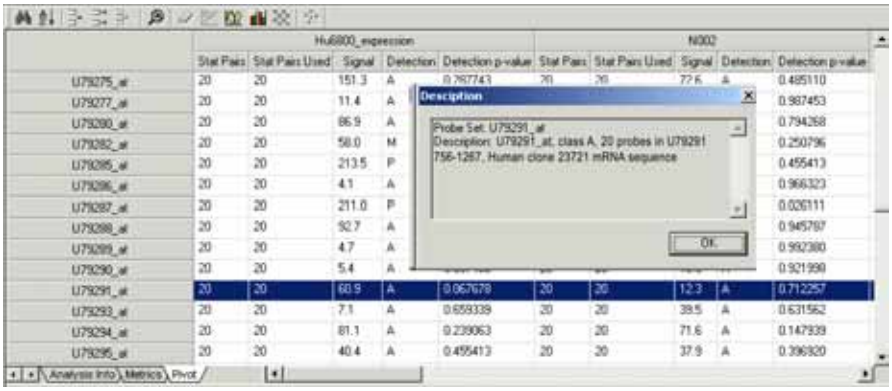


Figure 10.21
EAW, probe set Description dialog box

Obtaining Further Information

You can search for further information about a probe set at an Internet site.

1. In the metrics or pivot table:

   a. Right-click the probe set (row) of interest.

   b. Select **External Database →NetAffx (**or **Entrez)** from the shortcut menu that appears.

   ⇒ This starts the default Internet browser (for example, Microsoft® Internet Explorer or Netscape® Communicator) and automatically opens the selected Internet site.

Exporting the Pivot Table

You can export the pivot table as a text file (.txt) or Excel file (.xls).

1. If it is not already open, open the EAW (in the data tree, double-click the probe analysis data of interest).
2. Click the Pivot tab.
3. To export only a user-selected portion of the data table, highlight the cells of interest.
4. Click the **Save** button or select **File** →**Save As** from the menu bar.
   ⇒ The **Save As** dialog box appears **(Figure 10.22)**.

Figure 10.22
Save As dialog box

5. Choose a save option:
   - **Save All** places all of the pivot table in the text file.
   - **Save Selected** places only the selected pivot data in the text file.
6. Enter a name for the text file and confirm or select another directory for the text file.
7. Click **Save** to export the pivot table or selected pivot data.

Finding Probe Set Information

You can perform text searches in the EAW.

1. To start a text search, do one of the following:
   - click the **Find** button
   - select **Edit** →**Find** from the menu bar
   - right-click the metrics or pivot table and select **Find** in the shortcut menu that appears.

   ⇒ The Find dialog box appears **(Figure 10.23)**.

Figure 10.23
Find dialog box

2. Enter the text string for the search (up to 256 alphanumeric characters).
3. Choose the **Match** or **Direction** search option, then click **Find Next**. Click **Find Next** again to continue the search.

Note

*The **Find** command finds all strings that match the text string for the search. For example, using the **Find** command to search for the text string biob would find AFFX-BioB-5 as well as other occurrences of BioB.*

Sorting Probe Set Information

You can sort the metrics or pivot table by any category of data displayed in the table.

Single Column Sort

1. In the EAW, click the Metrics or Pivot tab.
2. Right-click the column header of interest and click **Sort Ascending** or **Sort Descending** in the shortcut menu that appears.

Multiple Column Sort

1. In the EAW, click the Metrics or Pivot tab.
2. Click the **Sort** button. Alternatively, select **Edit →Sort** from the menu bar.

   ⇒ The Sort dialog box appears **(Figure 10.24)**.

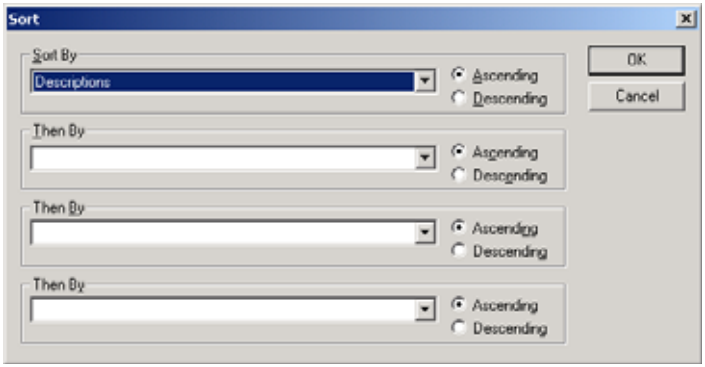


Figure 10.24
Sort dialog box

3. Select the desired sort category from the first (top) drop-down list and choose an ascending or descending sort order.

   Up to three additional sort categories may be specified.
4. Click **OK** when finished specifying the sort.

   The sort category and order are displayed at the bottom of the EAW.

Reordering or Removing Probe Set Information

Reordering Columns in the Metrics or Pivot Table

1. In the Metrics or Pivot tab, click the column header of interest
2. Use the drag-and-drop method to move the column to a new position in the table.

Reordering Rows in the Metrics or Pivot Table

Use the **Edit** commands in the menu bar to remove or reorder tabular data in the metrics or pivot table:

Edit →Move Selected to Top	Moves a selected row(s) to the top of the metric or pivot table.
Edit →Remove Analyses	When the pivot table contains only one analysis, removes the analysis from the table (equivalent to closing the probe analysis data). When the pivot table includes more than one analysis, this displays an Open dialog box that enables you to select the analyses you want to remove from the table.
Edit →Select All	Highlights all rows in the metric or pivot table.

Hiding Probe Set Information

You can hide tabular data in the metrics or pivot table. Use the View commands in the menu bar to hide (unhide) selected rows or all of the tabular data.

Alternatively, right-click the metrics or pivot table to display a shortcut menu of hide commands:

View →Hide Selected	Hides the currently selected row(s) in the metrics or pivot table. The status bar at the bottom of the EAW displays the remaining number of rows in the table.
View →Hide Unselected	Hides all unselected rows in the metrics or pivot table. The status bar at the bottom of the EAW displays the remaining number of rows in the data table.
View →Unhide All	Displays all previously hidden rows at the bottom of the data table.

Expression Analysis Measured Images

A measured image displays the probe set hybridization intensity data (from the image data).

Displaying Measured Images

1. Click the desired probe set name(s) in the metrics or pivot table.

   To select adjacent probe set names, press and hold the **Shift** key and click the first and last probe set name in the selection. To select non-adjacent probe set names, press and hold the **Ctrl** key while you click the desired names.

2. Click the **Image** button . Alternatively, select **Graphs →Measured Images** from the menu bar.

   ⇒ The graph pane (Intensity tab) opens and displays the measured image and the intensity signal range for the selected probe set **(Figure 10.25)**.

   When an additional image is created, the graph pane automatically scrolls to the new image. Use the scroll bar to view all of the measured images created during a session. See *Working with Windowpanes & Columns,* on page 624 for information about resizing the graph pane.

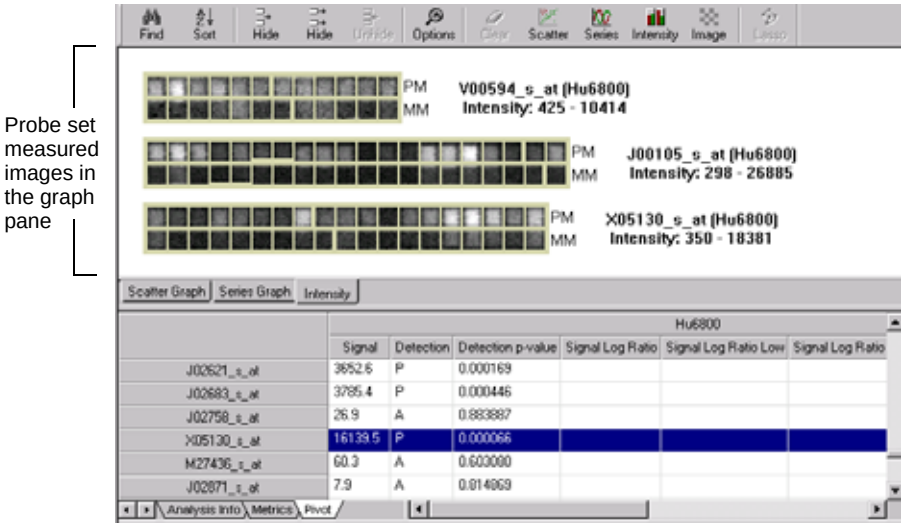


Figure 10.25
EAW, measured images in the graph pane (top)

Clearing Measured Images

You can remove all or selected images from the graph pane.

1. To remove all measured images from the graph pane, right-click the graph pane and select **Clear All Graphs** from the shortcut menu that appears. Alternatively, select **Graph →Clear All Graphs** from the menu bar.

2. To remove selected images from the graph pane:

   a. Double-click the measured image.

   b. Right-click the image and select **Clear Selected Graphs** from the shortcut menu that appears or select **Graph →Clear Selected Graphs** from the menu bar.

Expression Analysis Graphs

GCOS plots three types of expression analysis graphs (**Table 10.2**). The graphs are displayed in the graph pane that appears at the top of the EAW **(Figure 10.26)**.

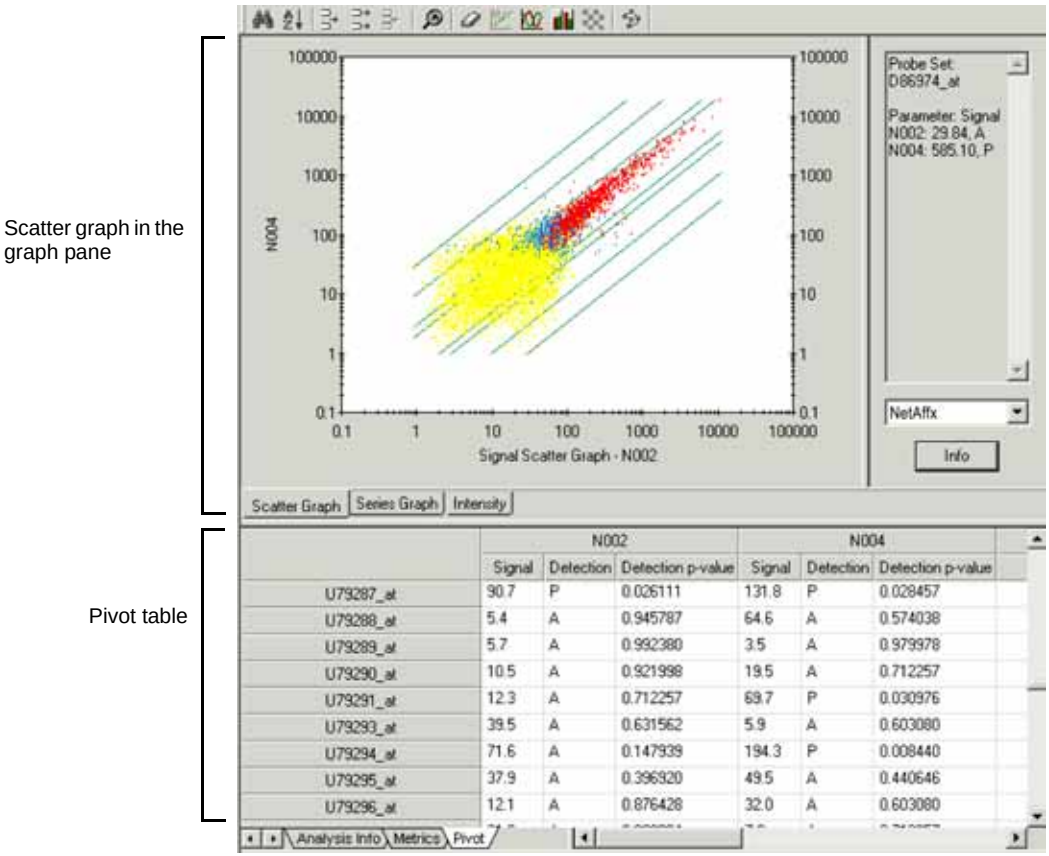
Table 10.2
Expression analysis graphs

Expression Graph Type	Plots...
Scatter	A user-specified metric for all probe sets from two experiments (of the same probe array type) using a traditional scatter plot.
Series	A user-specified metric for each probe set on the array in a bar or line graph format.
Intensity	The intensities of the probe pairs in a probe set using a bar graph format.

The graph pane has three tabs: Scatter Graph, Series Graph, and Intensity. Click the graph pane tabs to toggle the display among the different graph types. See *Working with Windowpanes & Columns*, on page 624 for information about resizing the graph pane.

Note

The intensity graph and images functions are unavailable until a probe set(s) is highlighted in the metrics or pivot table.



	N002			N004		
	Signal	Detection	Detection p-value	Signal	Detection	Detection p-value
U79287_at	90.7	P	0.026111	131.8	P	0.028457
U79288_at	5.4	A	0.945787	64.6	A	0.574038
U79289_at	5.7	A	0.992380	3.5	A	0.979978
U79290_at	10.5	A	0.921998	19.5	A	0.712257
U79291_at	12.3	A	0.712257	69.7	P	0.030976
U79293_at	39.5	A	0.631562	5.9	A	0.603080
U79294_at	71.6	A	0.147939	194.3	P	0.008440
U79295_at	37.9	A	0.396920	49.5	A	0.440646
U79296_at	12.1	A	0.876428	32.0	A	0.603080

Figure 10.26
Expression Analysis window (EAW), Scatter graph tab of the graph pane (top) and Pivot tab (bottom)

Scatter Graph

The scatter graph plots user-selected expression data for all probe sets from one or more pairs of analyses using a traditional scatter plot **(Figure 10.26)**. Each point in the scatter graph represents a probe set common to both experiments and is defined by the intersection of the analysis result value on the x and y axes for the common probe set. The scatter graph displays fold change lines (four pairs) that help identify probe sets in the comparison that change expression levels.

Plotting a Scatter Graph

1. Click the Scatter graph button . Alternatively, select **Graph** → **Scatter Correlation Graph** from the menu bar.

   ⇒ The Scatter Graph dialog box displays the pivot table columns available for the scatter graph **(Figure 10.27)**.

✔ Note

For the scatter plot, select pairs of analyses that are derived from the same probe array type. The order of the analyses is critical. For example, the analyses from a multiple probe array set must be ordered identically along the x and y-axes since the scatter graph compares the first set of analysis outputs on the x-axis with the first set of analysis outputs on the y-axis (and so forth). If the analysis outputs are not in identical order, many probe sets will not be compared and plotted (only the probe sets in common such as the controls).

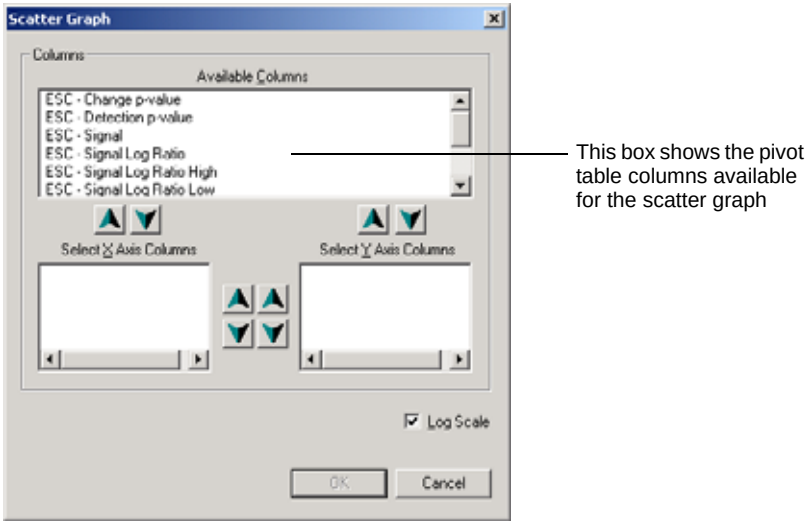


Figure 10.27
Scatter graph dialog box

2. To select a column for the x-axis, do either of the following:
   - Use the drag-and-drop method to move the column name from the **Available Columns** box to the **Select X Axis Columns** box **(Figure 10.27)**.
   - Highlight the column name in the **Available Columns** box, then click the down arrow (on the left side) to place the column in the **Select X Axis Columns** box.
3. To select a column for the y-axis, do either of the following:
   - Use the drag-and-drop method to move the column name from the **Available Columns** box to the **Select Y Axis Columns** box **(Figure 10.27)**.
   - Highlight the column name in the **Available Columns** box and click the down arrow (on the right side) to place the column in the **Select Y Axis Columns** box.
4. To change the order of columns, do either of the following:
   - Use the drag-and-drop method to move the column to the desired location in the list.
   - Highlight the column name, then use the up and down arrows located to the inside of the **Select Axis Columns** boxes.
5. To select linear scaling, remove the check mark from the **Log Scale** check box.

   Logarithmic scaling is the default.
6. Click **OK** to generate the scatter graph.

   ⇒ The graph pane (Scatter Graph tab) displays the scatter graph **(Figure 10.26)**.

Working with the Scatter Graph

Right-click the scatter graph to display a shortcut menu of commands that include:

Points Displays the absolute call combinations and the color assigned to each. The scatter graph displays the check marked items. Click an item to remove or add a check mark. The scatter graph is immediately updated.

Copy Graph	Copies the Scatter graph to the system clipboard.
Close Graph Pane	Closes the graph pane. Graphs created during the session are not saved.
Full Out Zoom	Restores the graph to the original magnification view.
Redraw Graph	Redraws the scatter graph so that it plots only the points that correspond to rows displayed in the pivot table. Points that correspond to hidden rows are not plotted.
Select Highlighted Points	Selects rows in the Pivot and Metric tables that correspond to points selected (*roped*) in the Scatter graph.
Options	Displays the Analysis Options dialog box.

Magnifying the Graph

1. Press and hold the **Shift** key while you use the click-and-drag method to draw a rectangle over the graph area of interest **(Figure 10.28)**.
2. Release the mouse key.

   ⇒ This magnifies the area selected by the rectangle **(Figure 10.29)**.
3. To zoom out and restore the graph, right-click the graph and select **Full Out Zoom** from the shortcut menu that appears.

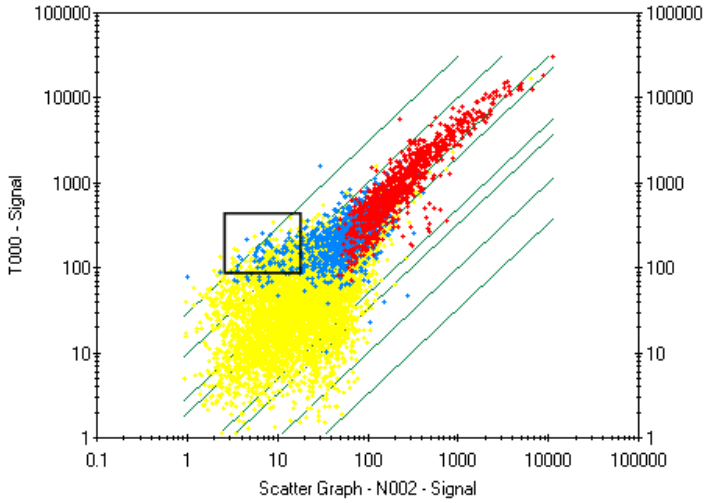


Figure 10.28
Scatter graph, rectangle selects an area to magnify

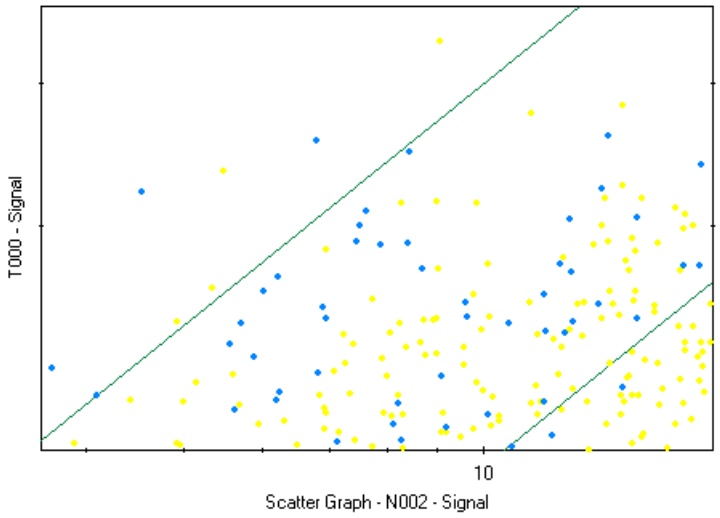


Figure 10.29
Magnified area in the scatter graph

Displaying Probe Set and Gene Information

1. Click a point in the scatter graph.

   ⇒ This displays the probe set name, parameter value, detection call, and a brief description of the probe set **(Figure 10.30)**. The corresponding row in the pivot table is highlighted.

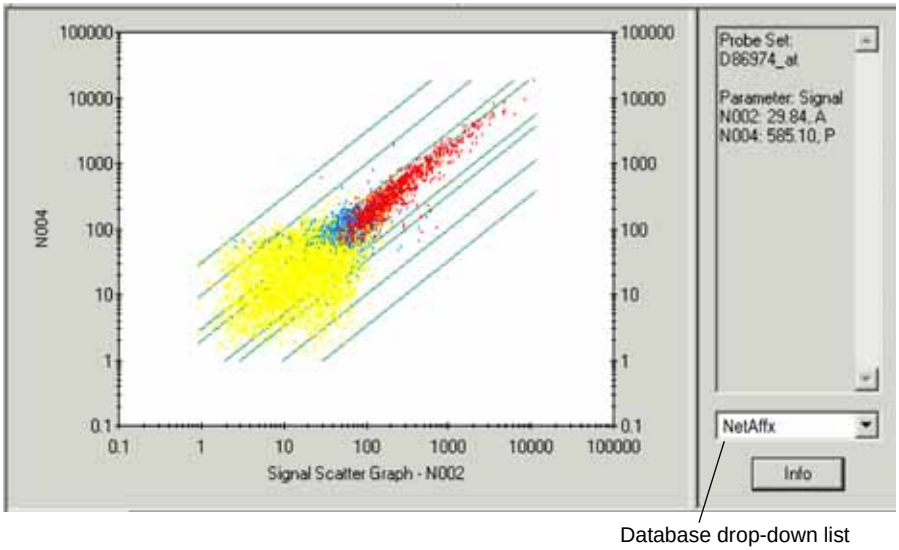


Figure 10.30
Scatter graph, click a point to display corresponding probe set and gene information

2. To obtain further gene information, select a database from the drop-down list and click **Info**.

   ⇒ The Internet browser window opens and displays information for the selected gene **(Figure 10.31)**.

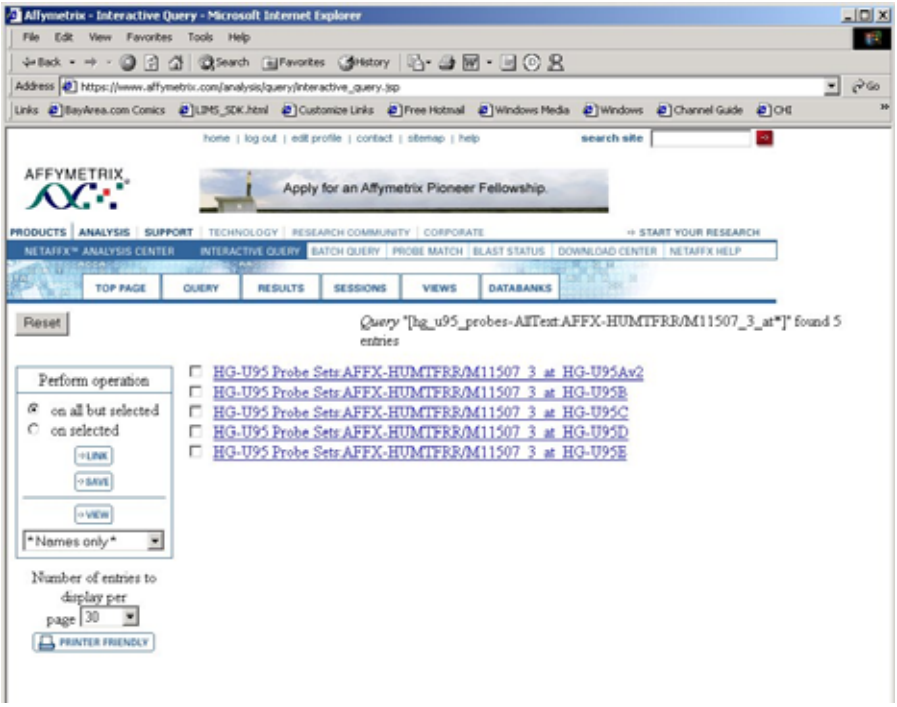


Figure 10.31
Internet browser window

Selecting Points in the Scatter Graph

1. Click the **Lasso** button . Alternatively, select **Graph →Lasso Points** from the menu bar.

   ⇒ The mouse pointer changes to a cross hairs (+) when it is positioned over the scatter graph

2. To rope points of interest, position the cross hairs near the group of points, then do either of the following:

   - Use the click-and-drag method to draw a circle around the points, then release the mouse button near the starting point.
   - Click the mouse, move it to draw a line segment, then click the mouse again to start drawing a new line segment. Repeat until you

return the cross hairs to the starting point and the line segments enclose the points of interest.

⇒ This completes the lasso operation **(Figure 10.32)**.

The scatter graph displays the selected points in orange. The metrics and pivot tables highlight the corresponding probe sets.

TIP *Press the **Esc** key to cancel the lasso operation.*

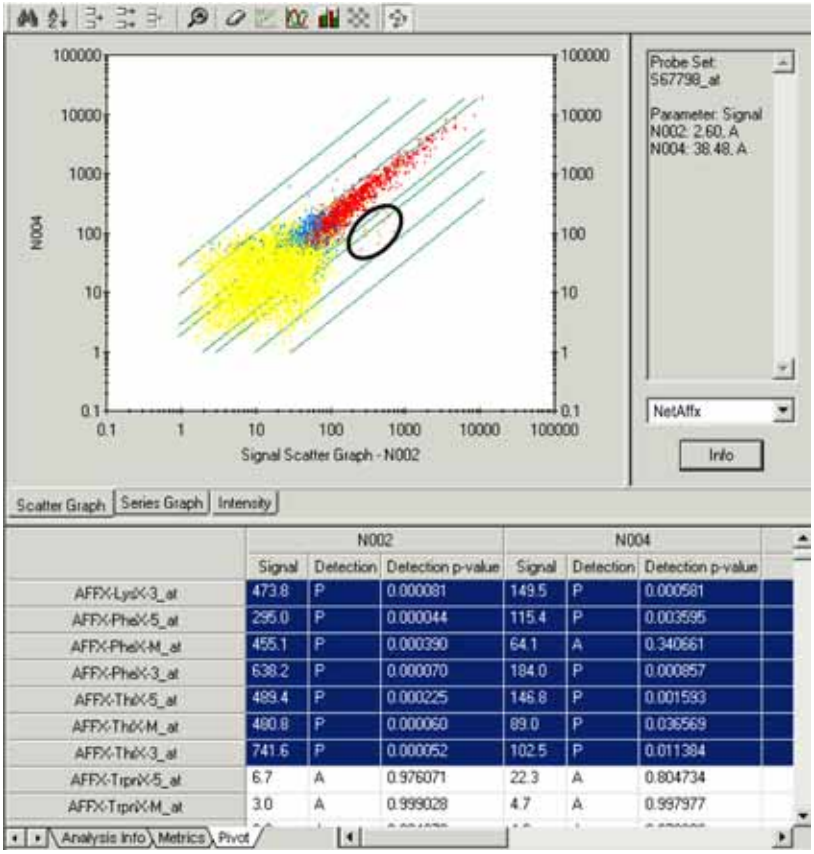


Figure 10.32
Roping points in the scatter graph highlights the corresponding probe set rows in the metrics and pivot tables

TIP

*After you select points in the scatter graph, hide the unselected rows in the pivot table, then use the **Redraw Graph** command to display only the selected points in the scatter graph.*

Hiding Points in the Scatter Graph

You can hide points in the scatter graph according to detection or Change call (if the scatter graph points are color coded using the **Color by Detection/Absolute Call or Color by Change/Difference Call** option, see *Point Options* on page 203.

1. Right-click the scatter graph and select **Points** from the shortcut menu that appears.

   ⇒ This displays a key for the scatter graph point colors **(Figure 10.33)**.

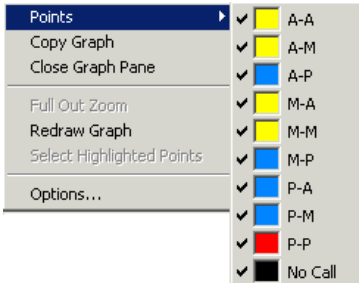


Figure 10.33
Scatter graph shortcut menu, point color combinations for detection calls

2. Click a point color combination to remove (or add) a check mark.

   ⇒ This hides (unhides) the selected points in the scatter graph.

Scatter Graph Options

You can modify some display features of the scatter graph. The Analysis Options dialog box displays the user-modifiable features **(Figure 10.34)**.

1. To open the Analysis Options dialog box, do one of the following:

   - Click the **Options** button .
   - Right-click the scatter graph and select **Options** from the shortcut menu that appears.

- Select **Analysis** →**Options** from the menu bar.

⇒ The Analysis Options dialog box appears **(Figure 10.34)**.

2. Click the Scatter Graph tab.

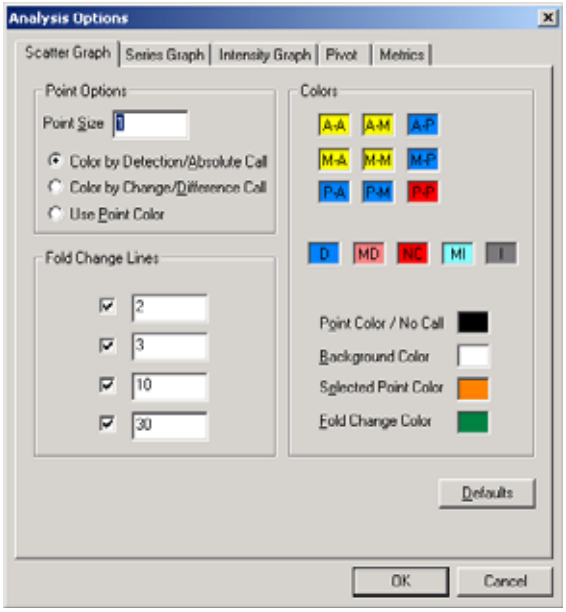


Figure 10.34
Analysis Options, Scatter Graph tab

Point Options

Point Size

Determines the size of the graph points. Enter a larger point size number for easier viewing, but use a smaller point size for high resolution graphs.

Color by Detection/ Absolute Call

Displays the graph points using the colors assigned to the single-array call combination of the x and y-axis analyses. There are nine possible call combinations:

	Absent in Y	Marginal in Y	Present in Y
Absent in X	A-A	M-A	A-P
Marginal in X	M-A	M-M	M-P
Present in X	P-A	P-M	P-P

TIP *Color coding the combinations can help quickly determine whether a highly expressed gene in one experiment was present or absent in another experiment.*

Note *If the call is No Call in either analysis, the point is displayed using the No Call Point Color (the default is black).*

Color by Change/Difference Call Displays the data points using the colors assigned to the comparison analysis call of the y-axis analysis. There are five possible calls: Decrease (D), Marginal Decrease (MD), No Change (NC), Marginal Increase (MI), and Increase (I).

Use Point Color Displays points using the color assigned to the Point Color.

Fold Change Lines

The default fold change lines are defined in pairs: y = 2x and y = 1/2x, y = 3x and y = 1/3x, y = 10x and y = 1/10 x, y = 30x and y = 1/30x.

The fold change lines (four pairs) are drawn using the values entered in the edit boxes. Only integer values may be entered. Removing a check mark turns off the display of that pair of fold change lines.

Colors

You can change the colors assigned to the:

- single-array analysis call categories
- comparison analysis call categories
- scatter graph points

- graph background
- selected (roped) scatter graph points
- fold change lines

1. In the Analysis Options dialog box **(Figure 10.34)**, click the color square associated with the item you want to change.

   ⇒ This displays the color palette **(Figure 10.35)**.

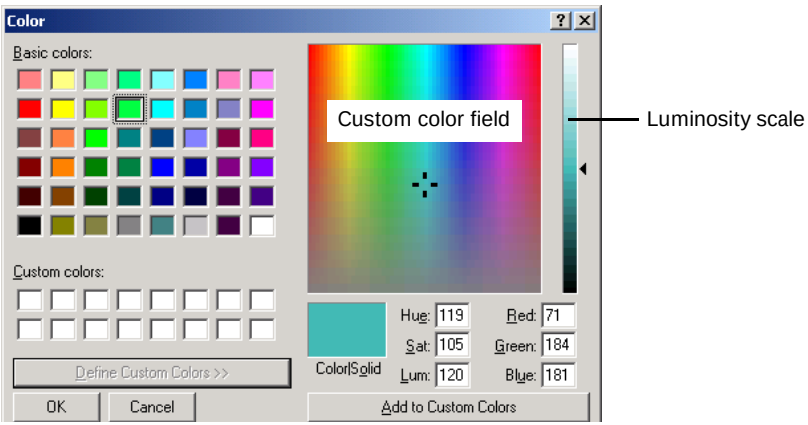


Figure 10.35
Color palette

2. To select a predefined color, click one of the basic colors.
3. To define a custom color, click **Define Custom Colors**, then use the click-and-drag method to move the cross hairs in the custom color field. Adjust the color brightness using the luminosity scale to the right. When finished, click **Add to Custom Colors** to apply the color.
4. Click **OK** to close the color palette.

   The color choices are saved on a per user basis.

Defaults

Click **Defaults** to return all analysis option settings to the factory set defaults.

Series Graph

The series graph plots a line or bar graph of user-selected expression data for all analyses or for all probe sets of analyses open in the EAW (**Figure 10.36** and **Figure 10.37**). The line graph format is the default.

Note *Hidden probe sets are not included in the series graph.*

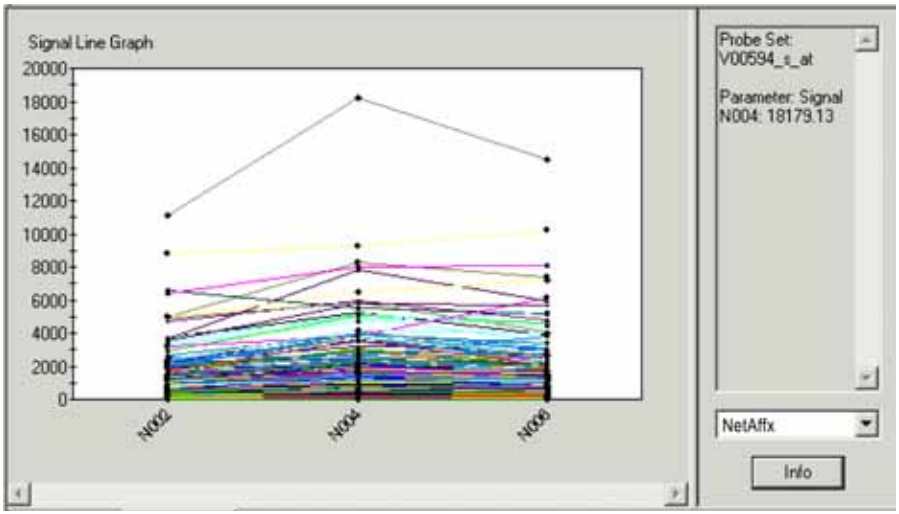


Figure 10.36
Series line graph, signal

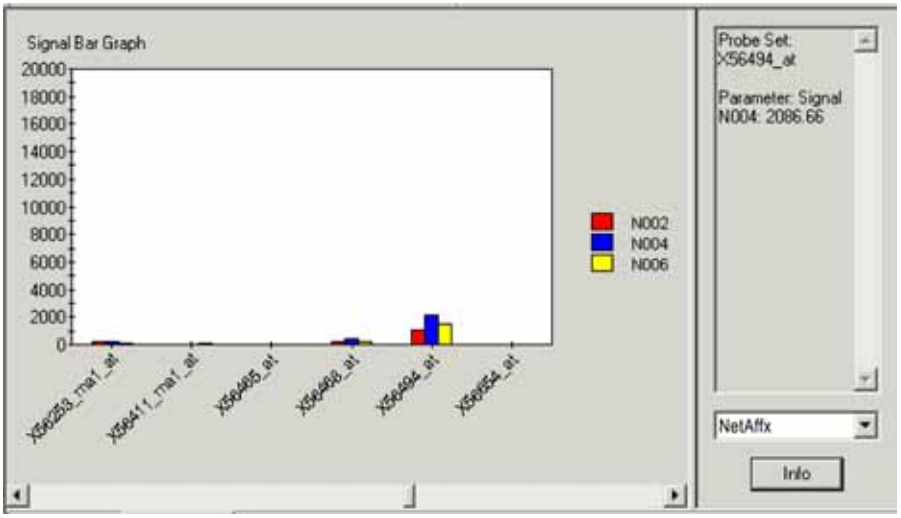


Figure 10.37
Series bar graph, signal

Plotting a Series Graph

1. Click the **Series Graph** button . Alternatively, select **Graph →Series Graph** from the menu bar.

   ⇒ The Series Graph dialog box appears **(Figure 10.38)**.

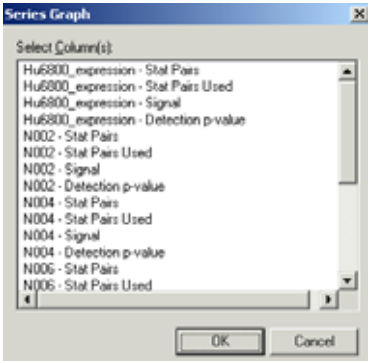


Figure 10.38
Series Graph dialog box

2. Select the pivot table columns for the series graph.

   ⇒ This generates the series line graph for the probe sets displayed in the data tables **(Figure 10.36)**.

   The line graph format is the default. For information on how to change the bar graph format, see *Series Graph Options* on page 208.

Working with the Series Graph

1. Place the mouse arrow over a probe set name, data point, or bar to view a pop-up tool tip that displays the analysis or probe set name and parameter value.
2. Right-click the series graph to display a shortcut menu of commands that include:

Copy Graph	Copies the graph to the system clipboard.
Close Graph Pane	Closes the graph pane (graphs created during the session are not saved).
Options	Displays the Analysis Options dialog box.

Series Graph Options

Some display features of the series graph may be modified. The Analysis Options dialog box displays the user-modifiable features **(Figure 10.39)**.

1. To open the Analysis Options dialog box, do one of the following:
   - Click the **Options** button .
   - Right-click the scatter graph and select **Options** from the shortcut menu that appears.
   - Select **Analysis** →**Options** from the menu bar.

   ⇒ The Analysis Options dialog box appears **(Figure 10.39)**
2. Click the Series Graph tab and choose the **Bar Graph** or **Line Graph** option.

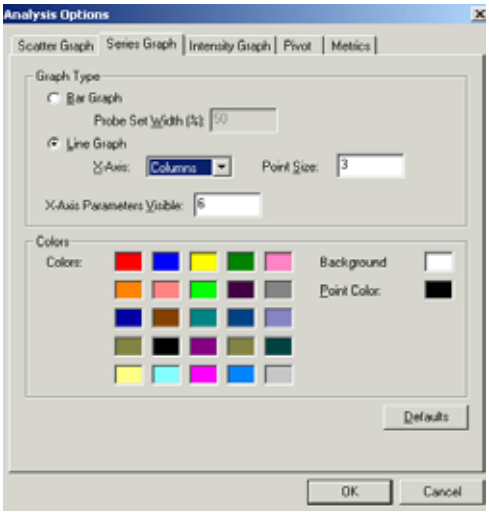


Figure 10.39
Analysis Options, Series Graph tab

Series Bar Graph Options

Probe Set Width (%)	Determines the graph bar width.
X-Axis Parameters Visible	The number of probe sets displayed on the x-axis within the viewable portion of the graph.

Series Line Graph Options

X-Axis	Select **Probe Sets** or **Columns** (from the **X-Axis** drop-down list) for display on the x-axis.
Point Size	Determines the size of the graph points.
X-Axis Parameters Visible	Specifies the number of probe sets or columns displayed on the x-axis within the viewable portion of the graph.

Colors

The color of the points or background in the series line graph may be changed.

1. In the Analysis Options dialog box **(Figure 10.39)**, click the **Point Color** or **Background** color square.

   ⇒ This displays the color palette **(Figure 10.40)**.

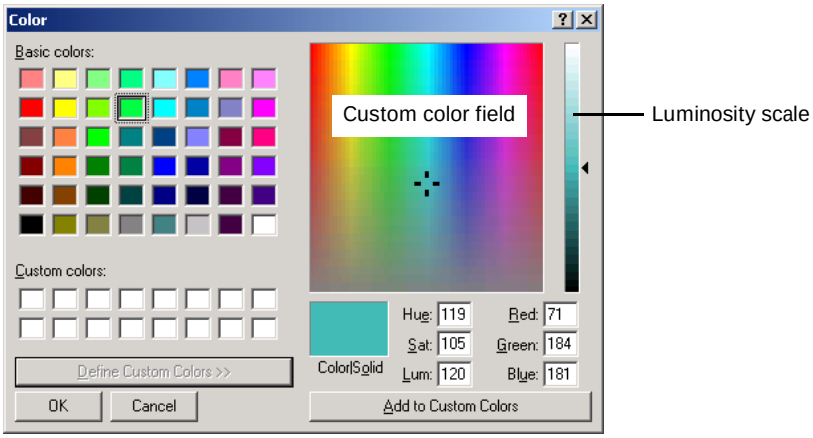


Figure 10.40
Color palette

2. To select a predefined color, click one of the basic colors.
3. To define a custom color, click **Define Custom Colors**, then use the click-and-drag method to move the cross hairs in the custom color field. Adjust the color brightness using the luminosity scale to the right. When finished, click **Add to Custom Colors** to apply the color
4. Click **OK** to close the color palette.

   The color choices are saved on a per user basis.

Intensity Bar Graph

The intensity bar graph displays the intensities of the probe pairs of a probe set in a bar graph format **(Figure 10.41)**. The intensity bar graph is a useful way to:

- evaluate the relative performance of the probe pairs in a probe set
- determine whether an image or probe mask has been applied to a probe pair

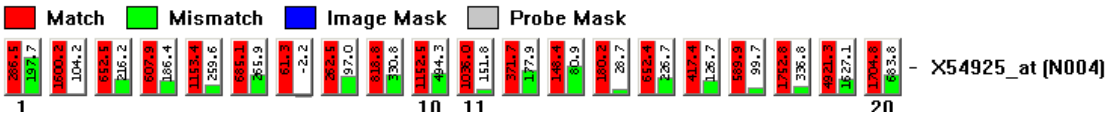


Figure 10.41
Intensity bar graph of probe set X54925_at (from experiment N004)

Plotting the Intensity Bar Graph

1. Highlight a row or cell in the metrics or pivot table.
2. To plot the intensity bar graph(s) for the highlighted probe set(s), do either of the following:
   - Click the **Intensity Bar Graph** button .
   - Select **Graph** →**Intensity Bar Graph** from the menu bar.
3. If necessary, use the scroll bar to view all of the intensity bar graphs created during a session.

Intensity Bar Graph Options

You can modify some display features of the intensity bar graph. The Analysis Options dialog box displays the user-modifiable features **(Figure 10.42)**.

1. To open the Analysis Options dialog box, do one of the following:
   - click the **Options** button
   - right-click the intensity bar graph, then click **Options** in the shortcut menu that appears
   - select **Analysis** →**Options** from the menu bar

   ⇒ The Analysis Options dialog box appears **(Figure 10.42)**
2. Click the Intensity Graph tab.

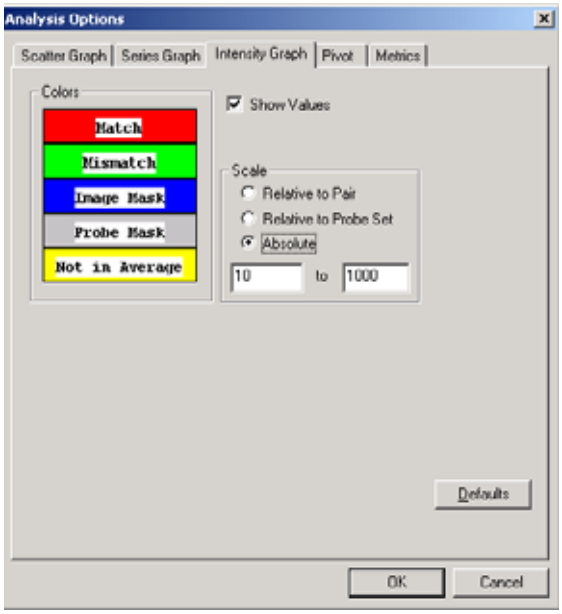


Figure 10.42
Analysis Options, Intensity Graph tab

Show Values

Choose the **Show Values** option to display numerical intensity values on the intensity bar graph.

Scale

Relative to Pair	Scales each probe pair set independently. The scale for each PM and MM pair is 0 →**max (PM, MM).**
Relative to Probe Set	Scales each probe pair relative to all probe pairs of a particular probe set. The scale is determined by all pairs of the probe set and ranges from **0 →max (all PM/MM probe pairs in the set).**
Absolute	Scales all probe pairs according to a user specified intensity range (for example, 10 to 1000 in **(Figure 10.42)**).

Colors

The color code specifies the colors for:

Match	Perfect match probe intensities
Mismatch	Mismatch probe intensities
Image Mask	Probe pairs included in an image mask
Probe Mask	Probe pairs included in a probe mask
Not in Average	Probe pairs excluded from the Average Difference calculation (Empirical expression algorithm only)

To change the colors specified by the color code:

1. Click the color square of the item you want to change (for example, **Match** in **Figure 10.42**).

   ⇒ This displays the color palette **(Figure 10.43)**.

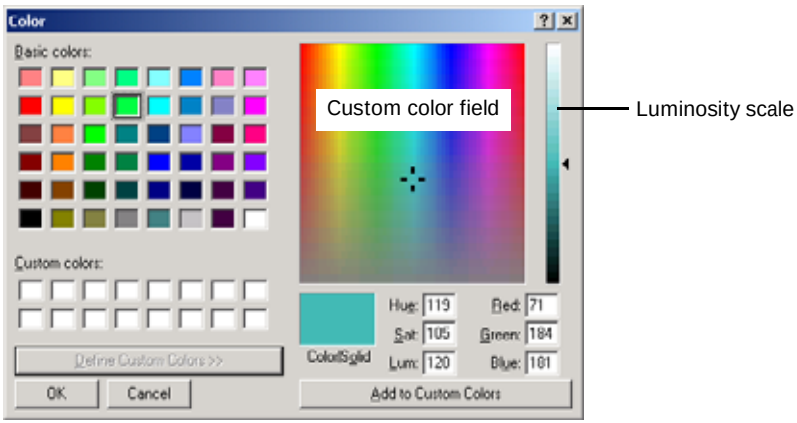


Figure 10.43
Color palette

2. To select a predefined color, click one of the basic colors.
3. To define a custom color, click **Define Custom Colors**, then use the click-and-drag method to move the cross hairs in the custom color field.

Adjust the color brightness using the luminosity scale to the right. When finished, click **Add to Custom Colors** to apply the color

4. Click **OK** to close the color palette.

   The color choices are saved on a per user basis.

Defaults

Click **Defaults** to restore the factory set defaults for all intensity bar graph options.

Clearing Intensity Bar Graphs

1. To remove all intensity bar graphs from the graph pane, right-click the graph pane and select **Clear All Graphs** from the shortcut menu that appears. Alternatively, select **Graph →Clear All Graphs** from the menu bar.
2. To remove user-selected intensity bar graphs from the graph pane:
   a. Double-click the graph(s) you want to remove
   b. Right-click the selected graph and select **Clear Selected Graphs** from the shortcut menu that appears. Alternatively, choose **Graph → Clear Selected Graphs** from the menu bar.

Expression Report

The expression report summarizes information about the expression analysis settings, algorithm settings, and probe set hybridization intensity data.

Generating an Expression Report

1. Confirm or change the user-modifiable report settings. See the next section *Expression Report Settings*.
2. To generate the report, do either of the following:
   - In the data tree, right-click the probe analysis data for the report and select **Report** from the shortcut menu that appears.
   - Select **File →Report** from the menu bar, then in the Report dialog box **(Figure 10.44)** that appears, double-click the probe analysis data for the report.

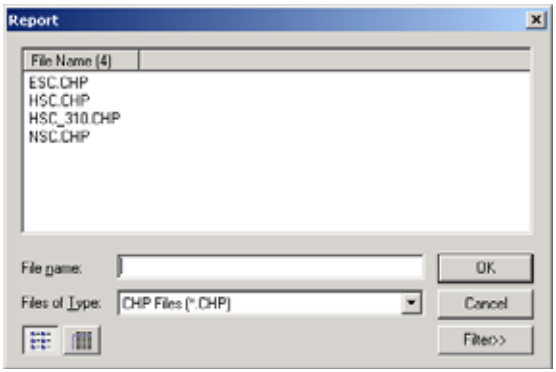


Figure 10.44
Report dialog box

⇒ This displays the expression report in the main display area. **Figure 10.45** and **Figure 10.46** show an example expression report.

3. To best view the expression report:

   a. Use Arial font (10 point) (select **View →Set Font** from the menu bar).

   b. Set the tab stops set at 10 (select **View →Set Tab Stops** from the menu bar).

✔ Note

A previously generated report will be overwritten. To save a previous report, rename it and save it under the new name. See the Saving an Expression Report, on page 224.

Report Type:	Expression Report
Date:	11:00AM 06/13/2001

Filename:	N006AS.CHP
Probe Array Type:	Hu6800
Algorithm:	Statistical
Probe Pair Thr:	8
Controls:	Antisense

Alpha1:	0.04
Alpha2:	0.06
Tau:	0.015
Noise (RawQ):	34.840
Scale Factor (SF):	1.000
Norm Factor (NF):	0.910

Baseline filename:	N004AS.CHP
Gamma1H:	0.0025
Gamma1L:	0.0025
Gamma2H:	0.0030
Gamma2L:	0.0030
Perturbation:	1.22
Baseline Noise (RawQ):	36.920
Baseline Scale Factor (SF):	1.000000

Background:				
	Avg: 826.54	Std: 18.25	Min: 788.10	Max: 869.20
Noise:				
	Avg: 26.71	Std: 1.06	Min: 25.00	Max: 30.40
Corner+				
	Avg: 24217	Count: 32		

Figure 10.45
Expression report, comparison analysis, Statistical Expression algorithm (page 1)

The following data represents probe sets that exceed the probe pair threshold and are not called "No Call".

Total Probe Sets:	7056	
Number Present:	1878	26.6%
Number Absent:	4988	70.7%
Number Marginal:	190	2.7%

Average Signal (P):	1202.8
Average Signal (A):	113.7
Average Signal (M):	294.0
Average Signal (All):	408.4

Number Increase:	94	1.3%
Number Decrease:	64	0.9%
Number MIncrease:	6	0.1%
Number MDecrease:	3	0.0%
Number No Change:	6889	97.6%

Number (A/M->P, MI/I)	23	0.3%
Number (P->A/M, MD/D)	12	0.2%
Number (P->P, MI/I)	63	0.9%
Number (P->P, MD/D)	41	0.6%
Number (A/M->A/M, MI/I)	14	0.2%
Number (A/M->A/M, MD/D)	14	0.2%
Number (P->P, NC)	1468	20.8%
Number (A/M->A/M, NC)	4941	70.0%

Number Increase:

(0<=SLR<1):	73
(SLR>=1):	27
(SLR>=2):	15
(SLR>=3):	2
(SLR>=4):	0

Number Decrease:

(0>=SLR>-1):	38
(SLR<=-1):	29
(SLR<=-2):	9
(SLR<=-3):	5
(SLR<=-4):	0

Housekeeping Controls:

Probe Set	Sig(5')	Det(5')	Sig(M')	Det(M')	Sig(3')	Det(3')	Sig(all)	Sig(3'/5')
HUMISGF3A/M97935	20.4	A	154.6	A	195.3	P	123.42	9.56
HUMRGE/M10098	10.6	A	30.7	A	34.9	A	25.42	3.29
HUMGAPDH/M33197	15088.9	P	14182.4	P	13387.5	P	14219.58	0.89
HSAC07/X00351	16005.2	P	26001.9	P	14967.8	P	18991.62	0.94
HUMTFRR/M11507	120.7	A	55.8	A	231.0	P	135.85	1.91
M27830	23.9	A	229.0	A	99.4	A	117.43	4.16

Spike Controls:

Probe Set	Sig(5')	Det(5')	Sig(M')	Det(M')	Sig(3')	Det(3')	Sig(all)	Sig(3'/5')
BIOB	53.0	A	23.4	A	82.0	A	52.78	1.55
BIOC	481.1	P			293.9	P	387.48	0.61
BIODN	970.7	P			3778.4	P	2374.53	3.89
CREX	5405.5	P			7817.0	P	6611.24	1.45
DAPX	2146.1	P	2199.2	P	2794.3	P	2379.85	1.30
LYSX	2217.5	P	4278.5	P	3546.6	P	3347.55	1.60
PHEX	2495.5	P	3087.7	P	3865.5	P	3149.54	1.55
THRX	3749.3	P	2322.5	P	3804.9	P	3292.25	1.01
TRPNX	18.9	A	8.9	A	14.5	A	14.12	0.77

Other Controls:

Probe Set	Sig	Det
AFFX-BIOB-5_AT	53.0	A
AFFX-BIOB-M_AT	23.4	A
AFFX-BIOB-3_AT	82.0	A
AFFX-BIOB-5_ST	44.6	A
AFFX-BIOB-M_ST	39.8	A
AFFX-BIOB-3_ST	17.8	A

Figure 10.46
Expression report, comparison analysis, Statistical Expression algorithm (page 2)

Expression Report Components

Statistical Expression Algorithm

Probe Pair Threshold

The minimum number of probe pairs a probe set must have in order for the probe set data to be included in the calculation of the report statistics

Alpha1 (α_1)

The significance level for the Detection *p*-value in an analysis. Alpha1 is a user-modifiable parameter that is set in the Parameters tab of the Expression Analysis Settings. See *Expression Analysis Settings*, on page 532.

If the probe set Detection *p*-value < alpha1, the call is present.

Alpha2 (α_2)

The second significance level for the Detection *p*-value in an analysis. Alpha2 is a user-modifiable parameter set in the Parameters tab of the Expression Analysis Settings. See *Expression Analysis Settings*, on page 532.

If the probe set Detection *p*-value ≥ alpha2, the call is absent. If alpha1 ≤Detection *p*-value < alpha2, the call is marginal.

Tau (τ)

Tau is a user-modifiable parameter that is set in the Parameters tab of the Expression Analysis Settings See *Expression Analysis Settings*, on page 532. Ideally, tau should be set to a value that is a little larger than the median of the discrimination scores of the probe sets whose targets are absent to avoid false detected calls.

Noise (Raw Q)

The degree of pixel-to-pixel variation among the probe cells used to calculate the background. See *Statistical Expression Algorithm*, on page 524.

Scale Factor (SF)

The scale factor specified in the Scaling tab of the Expression Analysis Settings dialog box or computed by the algorithm. See *Expression Analysis Settings*, on page 532.

TGT Value The user-specified target signal for scaling of the experiment probe array. The target signal is set in the Scaling tab of the Expression Analysis Settings dialog box. See *Expression Analysis Settings*, on page 532.

Norm Factor (NF) The normalization factor specified in the Normalization tab of the Expression Analysis Settings dialog box or computed by the algorithm. See *Expression Analysis Settings*, on page 532.

Gamma1H (γ_{1H}) The small significance level for Change calls at high intensities. Gamma1H is a user-modifiable parameter that is set in the Parameters tab of the Expression Analysis Settings. See *Expression Analysis Settings*, on page 532.

Gamma2H (γ_{2H}) The large significance level for Change calls at high intensities. Gamma2H is a user-modifiable parameter that is set in the Parameters tab of the Expression Analysis Settings. See *Expression Analysis Settings*, on page 532.

Gamma1L (γ_{1L}) The small significance level for Change calls at low intensities. Gamma1L is a user-modifiable parameter that is set in the Parameters tab of the Expression Analysis Settings. See *Expression Analysis Settings*, on page 532.

Gamma2L (γ_{2L}) The large significance level for Change calls at low intensities. Gamma2L is a user-modifiable parameter that is set in the Parameters tab of the Expression Analysis Settings. See *Expression Analysis Settings*, on page 532.

Perturbation A user-modifiable expression algorithm parameter that is set in the parameters tab of the Expression Analysis Settings. See *Expression Analysis Settings*, on page 532. Perturbation influences the *p*-value computed for a probe set in a comparison analysis.

Baseline Noise (Raw Q)	The degree of pixel-to-pixel variation among the probe cells used to calculate the background in the baseline probe array. See *Statistical Expression Algorithm*, on page 524.
Baseline Scale Factor (SF)	The scale factor specified for the baseline probe array in the Scaling tab of the Expression Analysis Settings dialog box or computed by the algorithm. See *Expression Analysis Settings*, on page 532.
Background	Minimum, maximum, average, and standard deviation of the background intensity calculated for the probe array.
Noise	The minimum, maximum, average, and standard deviation of the noise calculated for the probe array.
Corner+	The average cell intensity for the sense probe cells used in the grid alignment process.
Corner-	The average cell intensity of the antisense probe cells used in the grid alignment process.
Central+	The average cell intensity for the nine probe cells that comprise the cross at the center of a sense probe array.
Central-	The average cell intensity for the nine probe cells that comprise the cross at the center of an antisense probe array.
Total Probe Sets	The number of probe sets on the array that exceed the probe pair threshold and are not called *No Call*.
Average Signal	The average signal for all probe sets that exceed the probe pair threshold and are not called *No Call*.
Controls	The expression report includes the signal and call data for the probe sets that correspond to the housekeeping or spike control transcripts. Separate signal and call data are reported for the probe pairs specific to the 5’, middle (M), and 3’ regions of the control transcripts.
Sig(all)	The average signal for all control probe sets.
Sig(3′ /5′)	For a probe set, Sig(3′)/Sig(5′).

Expression Report Settings

The expression report has user-modifiable settings.

1. To view the user-modifiable settings, click **Settings** in the shortcut bar, then click **Expression Report**.

   ⇒ The Expression Report dialog box appears **(Figure 10.47)**.

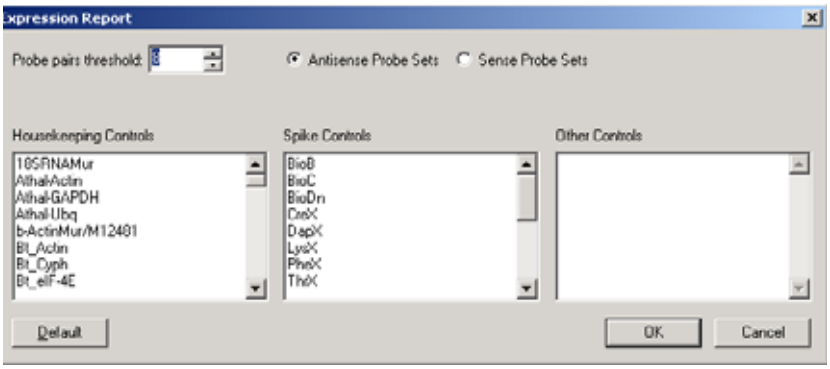


Figure 10.47
Expression Report dialog box

Probe Pair Threshold	The minimum number of probe pairs a probe set must have in order for the probe set data to be included in the calculation of the report statistics.
Antisense Probe Sets	Choose this option for probe arrays that have antisense control probes. (Refer to the probe array product insert.)
Sense Probe Sets	Choose this option for probe arrays that have sense control probes. (Refer to the probe array package insert.)

Housekeeping Controls

Housekeeping controls are housekeeping gene transcripts that are usually constitutively expressed in the sample. The transcripts serve as endogenous controls and are useful for monitoring the quality of the target.

The report includes the signal (Sig) for probe sets that are designed to be specific to the 5′, middle, or 3′ portion of the transcript. These data are informative about the reverse transcription and IVT steps in sample preparation.

Differences greater than three fold between the Sig 3′ and Sig 5′ (Sig 3′/5′) for a housekeeping control indicate the target may be degraded and should be prepared again.

■ To delete a housekeeping control from the expression report:

1. Double-click the probe set name.
2. Right-click probe set and select **Delete** from the shortcut menu that appears.

Spike Controls

Spike controls are transcripts that are added to or *spiked* in the sample. These are exogenous transcripts that are useful for monitoring assay procedures such as hybridization, washing, and staining.

■ To delete a spike control from the expression report:

1. Double-click the probe set name.
2. Right-click probe set and select **Delete** from the shortcut menu that appears.

Other Controls

You can choose any probe set on the array as a control. Enter the probe set name in the **Other Controls** box. The expression report includes the signal and detection call for user-specified controls.

Expression Report View Options

Find Feature

The Find feature searches the report.

1. Click the **Find** button or select **Edit →Find** from the menu bar.
   ⇒ The Find dialog box appears **(Figure 10.48)**.

Figure 10.48
Find dialog box

2. Enter the text for the search (up to 256 alphanumeric characters including spaces) in the **Find what** box, then click **Find Next** to view the first search result.

3. Click **Find Next** or the **Find Next** button to view each successive search result.

Editing an Expression Report

Edit commands for the expression report are available in the:

- Edit menu bar
- Report window toolbar
- Shortcut menu (right-click the report)

Replace Feature

The replace feature performs a text search and replaces the search item with specified text.

1. Select **Edit →Replace** from the menu bar.
   ⇒ The Replace dialog box appears **(Figure 10.49)**.

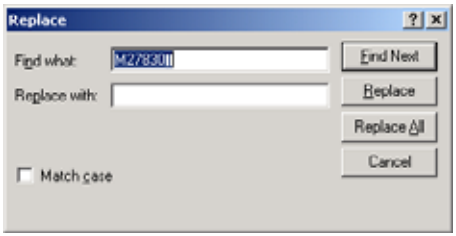


Figure 10.49
Replace dialog box

2. Enter the text for the search in the **Find what** box and enter the replacement text in the **Replace with** box.

3. Click **Find Next** to begin the search, then click **Replace** to replace the search item with the specified text. Repeat to find and replace successive instances of the search item. Alternatively, click **Replace All** to find and replace all occurrences of the search item in the report.

Saving an Expression Report

When GCOS generates a report for expression probe analysis data, it overwrites a previously generated report. To prevent a previous report from being overwritten, save it under a new name.

1. To open the report that you want to save under a new name, click the **Open** button.

   ⇒ The Open dialog box appears **(Figure 10.50)**.

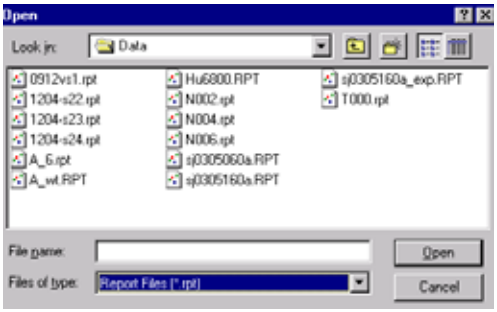


Figure 10.50
Open dialog box

2. Double-click the report name.

   ⇒ The report is displayed.

3. Select **File →Save As** from the menu bar.

   ⇒ The Save As dialog box appears **(Figure 10.51)**.

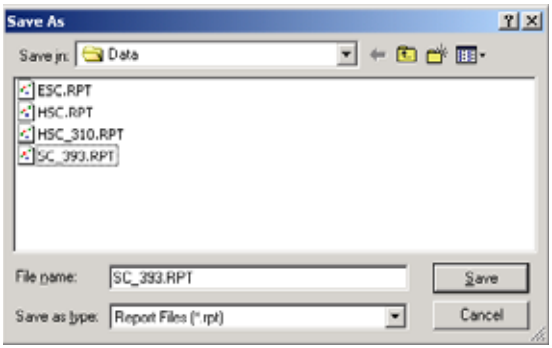
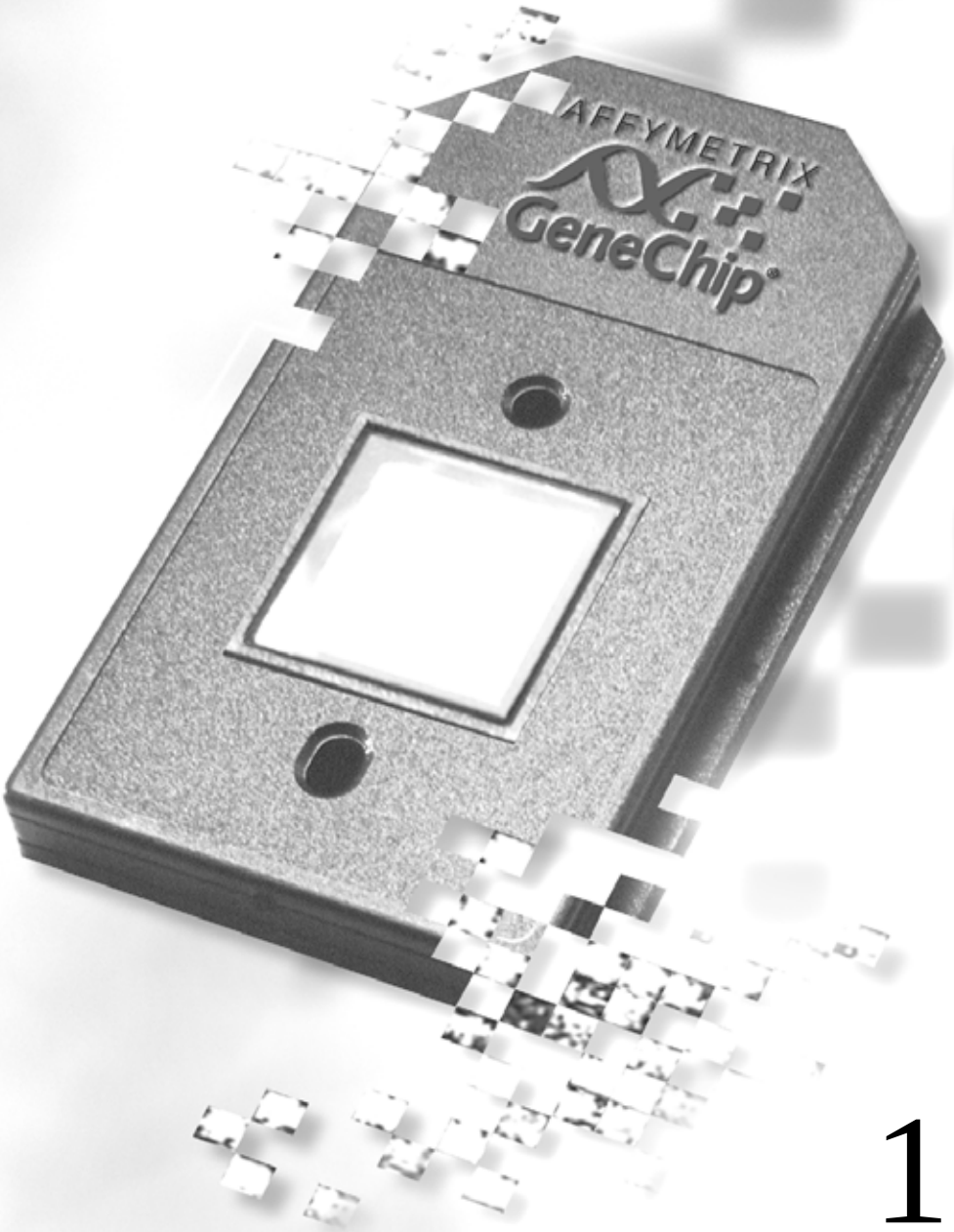


Figure 10.51
Save As dialog box

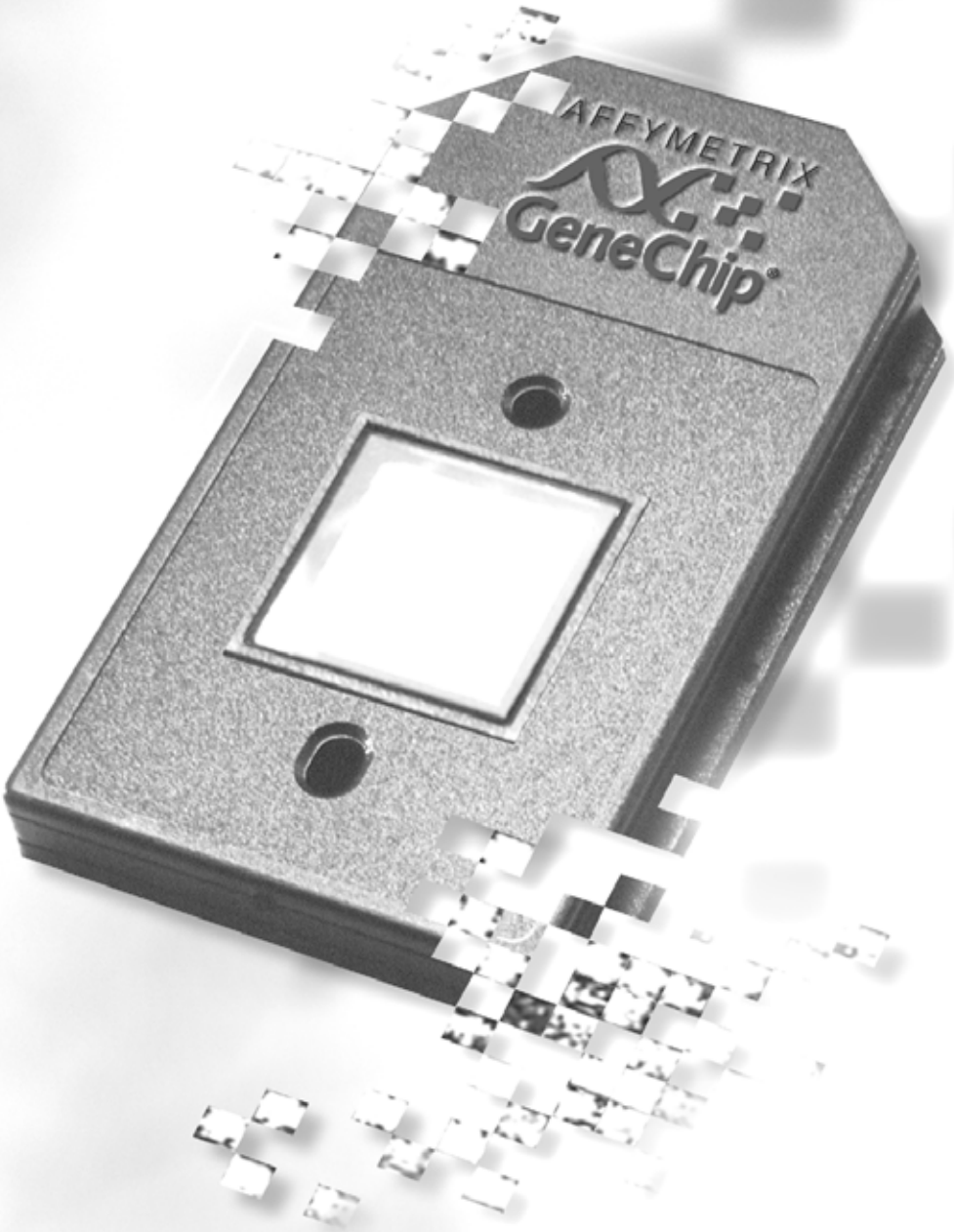
4. Click the report to be saved, then enter a new name for the report in the **File name** box.

5. Click **Save.**



11

Chapter 11



Batch Analysis

Batch analysis is a convenient way to analyze the cell intensity data from many different experiments and to generate probe analysis data with unattended operation.

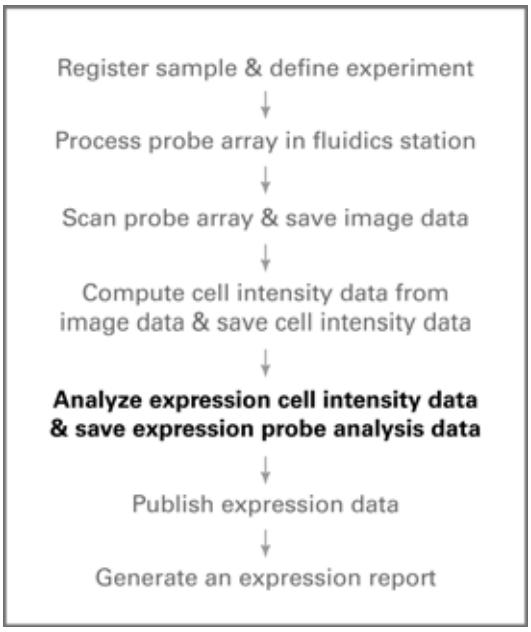


Figure 11.1
Assay & analysis flow chart

Opening the Batch Analysis Window

Do either of the following to open the Batch Analysis window:

- In the GeneChip Software shortcut bar, click **Batch Analysis** .
- Select **Run** →**Batch Analysis** from the menu bar.

  ⇒ The Batch Analysis window opens **(Figure 11.2)**.

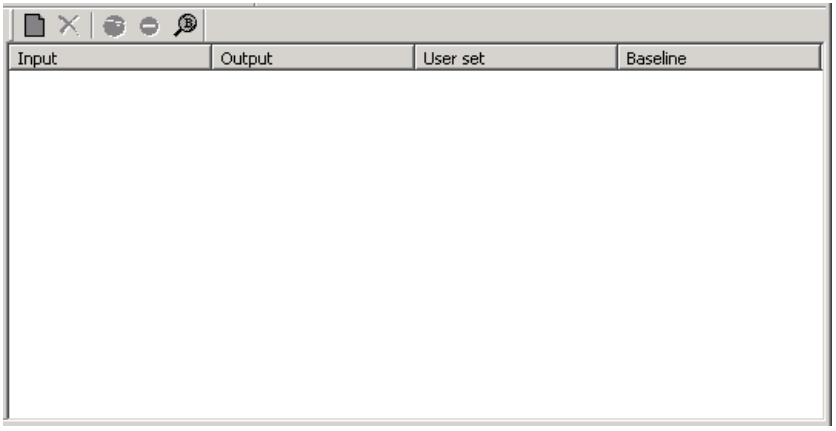


Figure 11.2
Batch Analysis window

Selecting Data for Batch Analysis

You may select cell intensity data or probe analysis data for batch analysis. If you select cell intensity data, GCOS automatically places the parent cell intensity data and baseline probe analysis data (if applicable) in the Batch Analysis window.

! CAUTION *Batch analysis will overwrite previous probe analysis data.*

Using the Drag-and-Drop Method

1. In the data tree, select the cell intensity data or probe analysis data that you want to analyze.

   To select adjacent items, press and hold the **Shift** key while you click the first and last item in the selection. To select non-adjacent items, press and hold the **Ctrl** key while you click the items.

2. Drag the selected data from the data tree to the Batch Analysis window **(Figure 11.3)**.

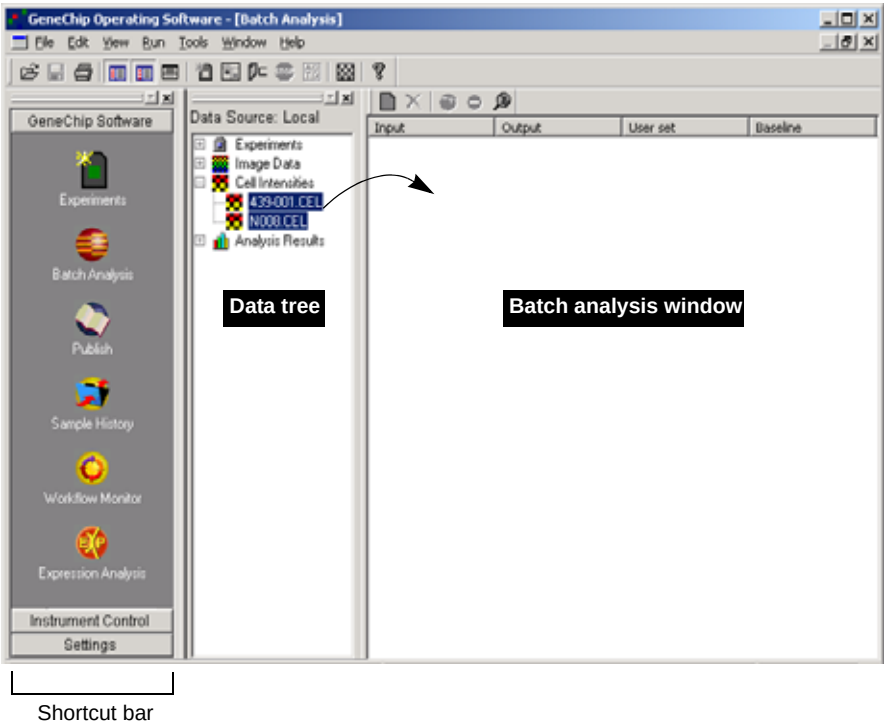


Figure 11.3
Batch Analysis window

Using the Toolbar or Menu Commands

1. Click the **Add** button or select **Edit** →**Add Item** from the menu bar.

   ⇒ The Add Cell/Chip Data dialog box appears **(Figure 11.4)**.

   Click **Details** to display experiment and sample information for the cell intensity or probe analysis data.

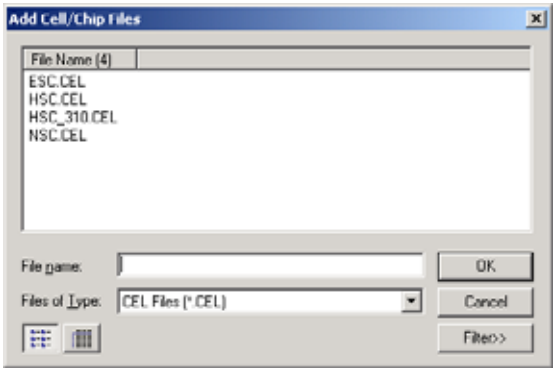


Figure 11.4
Add Cell/Chip Data dialog box

2. To filter the data in the Add Cell/Chip Data dialog box:

   a. Click **Filter**.

      ⇒ The advanced filter appears **(Figure 11.5)**.

   b. Make selections from the drop-down lists.

   c. Click **Apply**.

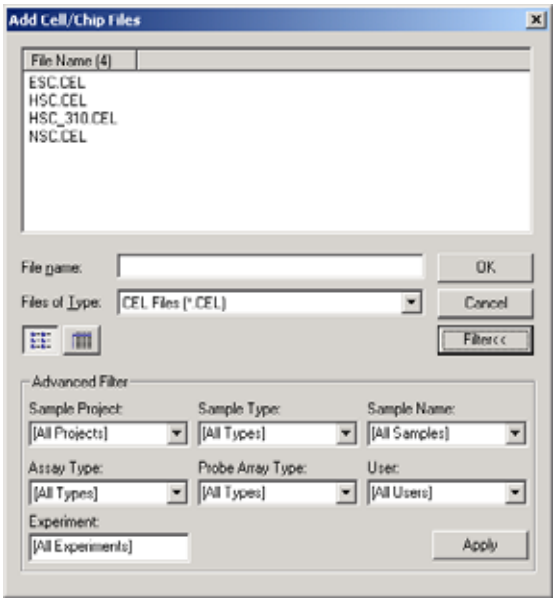


Figure 11.5
Add Cell/Chip Data dialog box, advanced filter

3. Select the data for the batch analysis, then click **OK**.

   To select adjacent items, press and hold the **Shift** key while you click the first and last item in the selection. To select non-adjacent items, press and hold the **Ctrl** key while you click the items.

   ⇒ The selected cell intensity data are added to the Batch Analysis window **(Figure 11.6)**.

! CAUTION *Batch analysis will overwrite previous probe analysis data.*

The Batch Analysis window displays previously analyzed cell intensity data in red to indicate that the existing probe analysis data will be overwritten. To reanalyze experiments without overwriting existing probe analysis data, specify a different name for the .chp from the batch analysis. See *Renaming Results from Batch Analysis* on page 238.

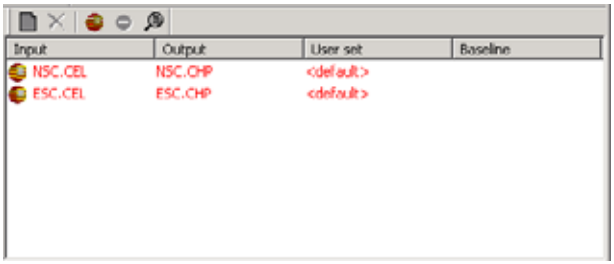


Figure 11.6
Batch Analysis window, cell intensity data (.cel) selected for batch analysis

Selecting a Userset for Expression Analysis

A userset specifies the expression analysis settings (scaling, normalization, probe masks, baseline experiment, values for user-modifiable Statistical Expression algorithm parameters). See *Userset Tab* on page 335 for more information about usersets.

Note

In a batch analysis, the settings for the default userset are specified in the Expression Analysis Settings dialog. The default userset is not the userset specified during experiment setup.

1. To select other than the default userset, double-click the **Userset** column.

   ⇒ This displays a drop-down list of available usersets **(Figure 11.7)**.

Note

If a userset with a baseline experiment is selected and the field under the baseline column is empty, the baseline experiment from the userset is used during the analysis.

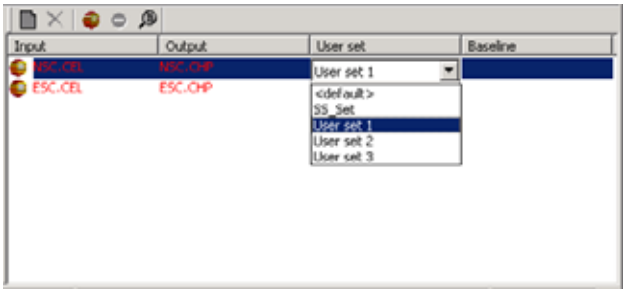


Figure 11.7
Batch Analysis window, drop-down list of usersets

2. Select a userset from the drop-down list. Alternatively, highlight the cell intensity data of interest, select **Edit →Select Userset** from the menu bar, then make a selection from the drop-down list.

Selecting a Baseline for a Comparison Expression Analysis

1. In the Batch Analysis window, double-click in the baseline column of the cell intensity data for the comparison expression analysis **(Figure 11.8)**.

   Alternatively, select the .cel of interest and choose **Edit →Select Baseline** from the menu bar.

   ⇒ The Select Baseline Data dialog box appears **(Figure 11.9)**.

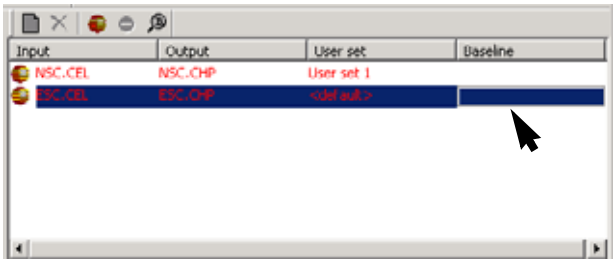


Figure 11.8
Batch Analysis window

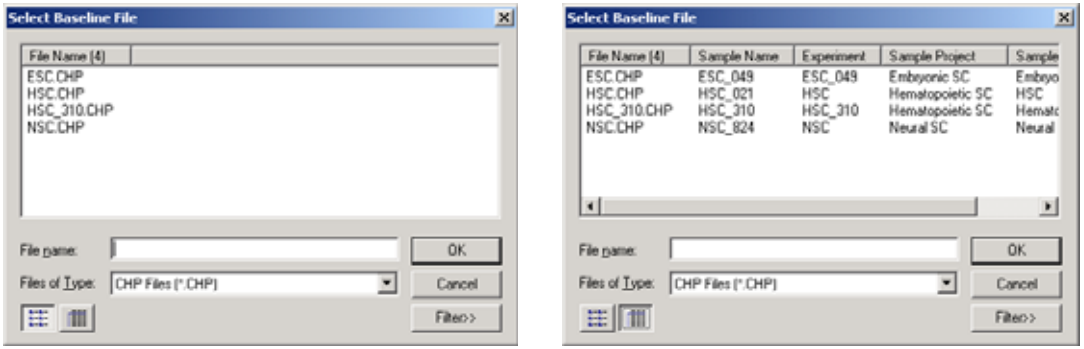


Figure 11.9
Select Baseline dialog box, List view (left) and Details view (right)

2. Click **Details** to display experiment and sample information for the data.
3. To filter the data in the Select Baseline dialog box:
   a. Click **Filter**.
      ⇒ This displays the advanced filter **(Figure 11.10)**.
   b. Make selections from the drop-down lists.
   c. Click **Apply**.

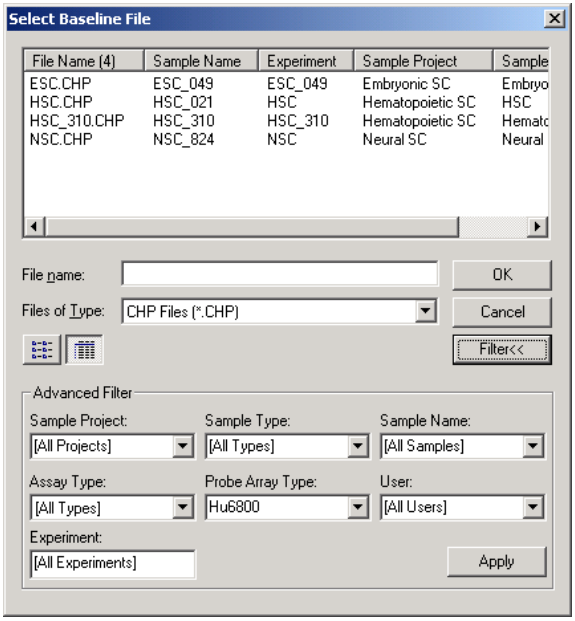


Figure 11.10
Select Baseline dialog box, advanced filter

4. Double-click the baseline data.

   ⇒ The Batch Analysis window displays the baseline data **(Figure 11.11)**.

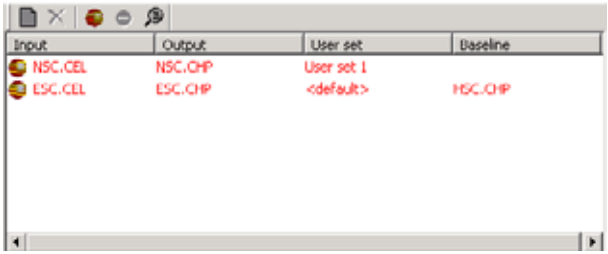


Figure 11.11
Batch Analysis window, baseline selected

5. To clear a baseline from the batch analysis window, right-click the baseline data and select **Clear Baseline** from the shortcut menu that appears.

   Alternatively, click the baseline experiment and select **Edit →Clear Baseline** from the menu bar.

Renaming Results from Batch Analysis

To reanalyze experiments without overwriting existing analysis output, rename the output .chp data from the batch analysis.

Renaming Results Automatically

If you select .cel data for batch analysis, GCOS can automatically rename all of the output .chp data by adding a user-specified prefix and/or suffix to the data name.

✔ Note *If you select .chp data for batch analysis, the prefix or suffix are not applied to the output name.*

1. Click the **Options** button or select **View →Options** from the menu bar.

   ⇒ The Batch Analysis Options dialog box appears **(Figure 11.12)**.

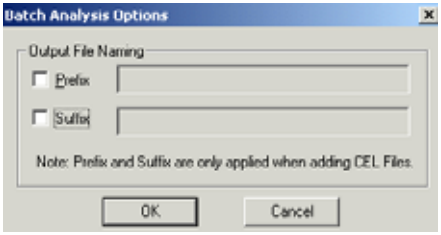


Figure 11.12
Output Naming Options dialog box

2. To add a prefix to the .chp name, choose the **Prefix** option and enter a prefix for the name in the associated box.

3. To add a suffix to the .chp name, choose the **Suffix** option and enter a suffix for the name in the associated box.
4. Click **OK**.
   ⇒ When cell intensity data are placed in the Batch Analysis window, the user-specified prefix and/or suffix is automatically added to the output analysis name.

Renaming Results Individually

You can individually rename an output results from the batch analysis.

Editing the Output Name

To edit the default name, do either of the following:

- Double-click the name in the Batch Analysis window and enter a new name.
- Select the cell intensity data of interest in the Batch Analysis window, select **Edit →Rename Output Data** from the menu bar, and enter a new name.

Adding a Prefix or Suffix to the Output Name

1. In the Batch Analysis window, use one of the following methods to select one or more outputs.
   - To select all data, right-click a name and click **Select All** in the shortcut menu that appears. Alternatively, select **View →Select All** from the menu bar.
   - To select adjacent data, press and hold the **Shift** key while you click the first and last name in the selection.
   - To select non-adjacent data, press and hold the **Ctrl** key while you click the names.
2. Right-click the selected outputs and select **Set Prefix/Suffix** from the shortcut menu that appears.

   Alternatively, click the selection and select **Edit →Set Prefix/Suffix** from the menu bar.

   ⇒ The Set Prefix/Suffix dialog box appears **(Figure 11.13)**.

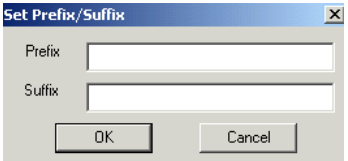


Figure 11.13
Set Prefix/Suffix dialog box

3. Enter a prefix and/or suffix, then click **OK**.

   ⇒ The prefix and/or suffix are added to the selected output names.

Removing Data from the Batch Analysis Window

To remove cell intensity data from the Batch Analysis window, do either of the following:

- Select the data name and click the **Remove** button .
- Select the data name, then select **Edit** →**Remove Item** from the menu bar.

Running a Batch Analysis

1. To run the batch analysis, click the **Analyze** button . Alternatively, select **Edit** →**Start Analysis** from the menu bar.

2. To stop a batch analysis in progress, click the **Stop** button . Alternatively, select **Edit** →**Stop Analysis** from the menu bar.

   The software completes the current analysis and cancels the remaining data in the batch analysis queue.

Resuming a Batch Analysis

If the batch analysis is interrupted before all analyses are completed, GCOS can resume the run and analyze the remaining .cel data in the batch.

To resume a batch analysis:

1. Open the Batch Analysis window.
   ⇒ The Batch Analysis window displays the remaining unanalyzed data.

2. Click the **Analyze** button . Alternatively, select **Edit →Start Analysis** from the menu bar.

Exporting a Batch File

The Batch Analysis window contents can be exported to a text file (tab delimited .txt). The batch file provides a record of the analysis and can be imported for a subsequent batch analysis.

1. Select **Edit →Export Batch** from the menu bar.
   ⇒ The Export Batch dialog box appears **(Figure 11.14)**.

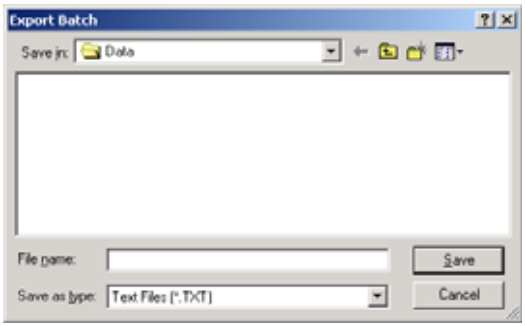


Figure 11.14
Export Batch dialog box

2. Enter a name for the batch file, then click **Save**.
   ⇒ The batch file (.txt) is created **(Figure 11.15)**.

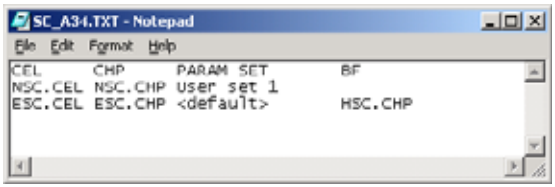


Figure 11.15
Batch file (.txt)

Importing a Batch File

GCOS can import a batch file (tab delimited .txt) that follows the format shown in **Figure 11.15**.

To import a batch file:

1. Open the Batch Analysis window.
2. Select **Edit →Import Batch** from the menu bar.
   ⇒ The Import Batch dialog box appears **(Figure 11.16)**.

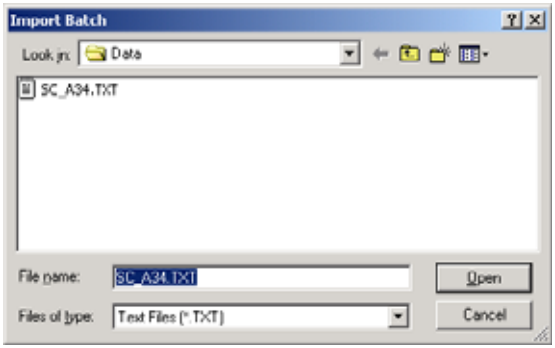
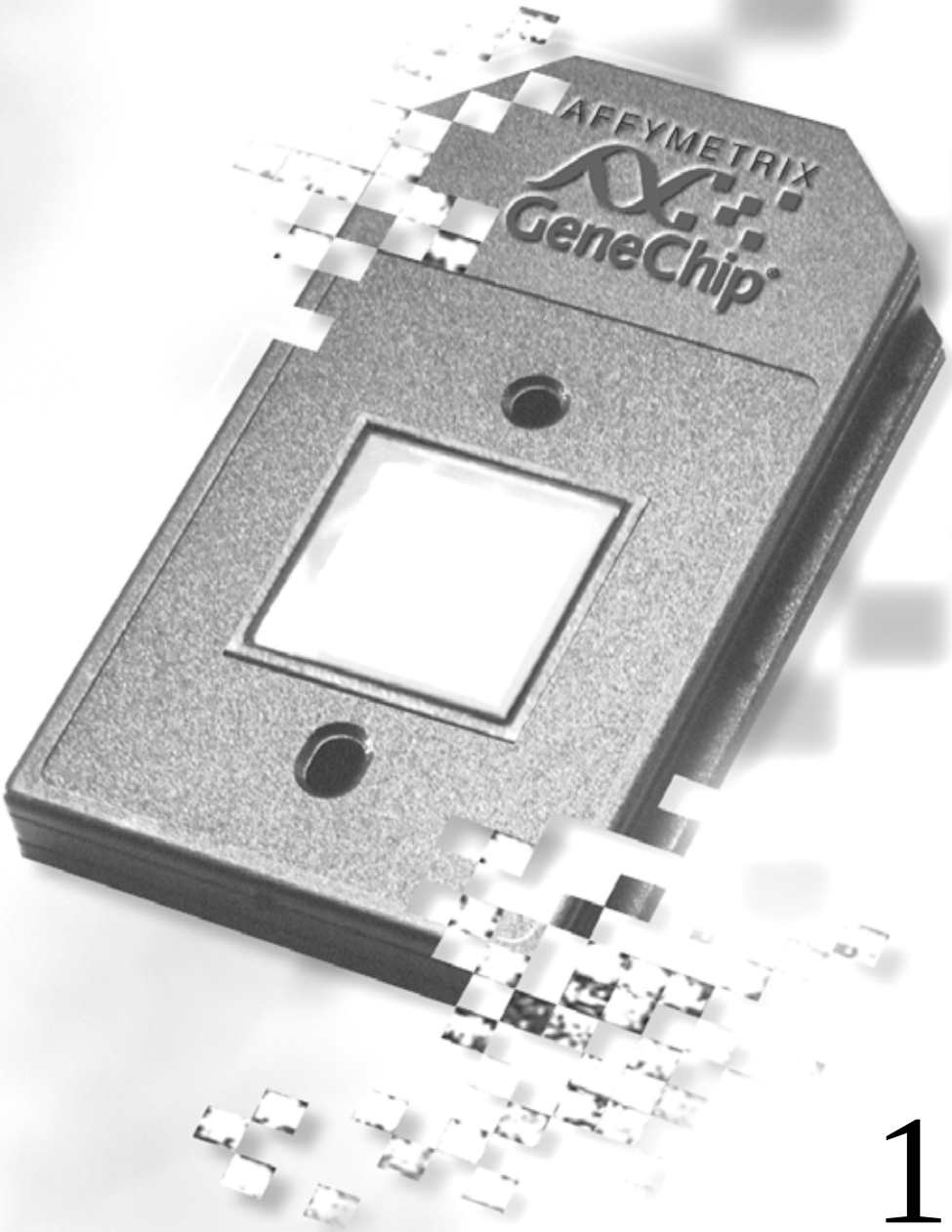


Figure 11.16
Import Batch dialog box

3. Double-click the batch file (.txt) you want to import.
   ⇒ The batch file is imported to the Batch Analysis window.



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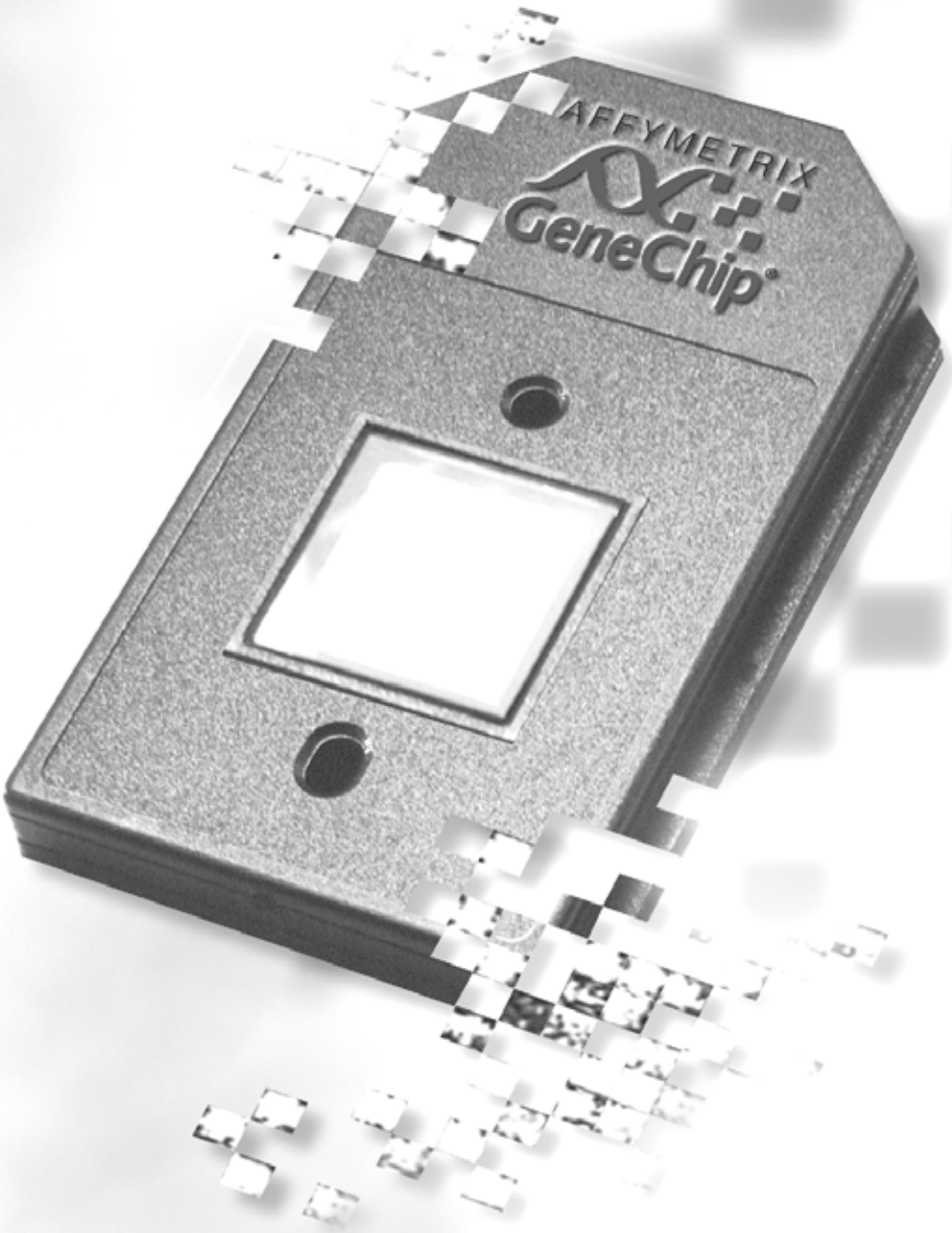
Chapter 12



Publishing Data

GCOS can copy expression sample and experiment data, including cell intensity data and probe analysis data from the process database to a publish database. This is called publishing. Published expression data can be queried by the Affymetrix® Data Mining Tool (DMT) software or several other third party analysis tools.

Figure 12.1
Assay & analysis flow chart

Selecting the Publish Database

1. In the GeneChip Software shortcut bar, click **Publish** . Alternatively, select **Run →Publish** from the menu bar.

   ⇒ This displays the publish window **(Figure 12.2)**.

   The top windowpane displays all publish databases. The bottom windowpane displays a status log of tasks.

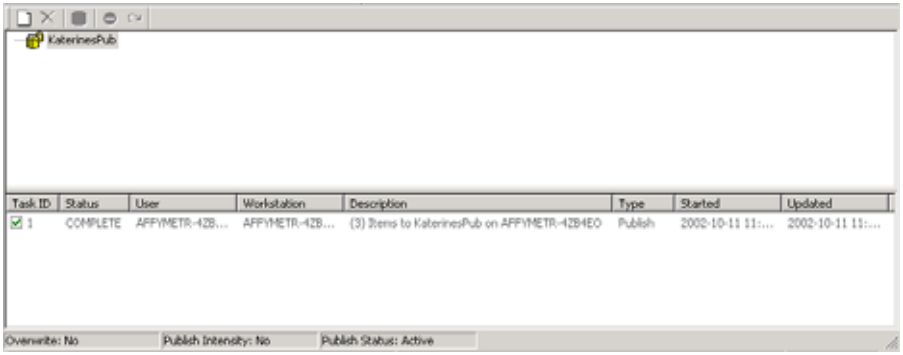


Figure 12.2
Publish window

2. Click the publish database destination for the data.

   A yellow icon indicates the currently selected database. A gray icon indicates an unselected database.

✔ Note *Each database requires a login password before publishing can proceed.*

Specifying the Task

A task consists of all data selected for publishing. Each experiment or type of data to be published is called a task item. **Table 12.1** shows the data that are published for each type of task item.

Table 12.1
Data published for different types of task items

Task Item	Data Published
Experiment	All cell intensity data associated with the experiment. All probe analysis data associated with the experiment.
Image data	All cell intensity data associated with the experiment. All probe analysis data associated with the experiment.
Cell intensity data	The associated experiment. The cell intensity data. All probe analysis data associated with the cell intensity data.
Probe analysis data	The associated experiment. The probe analysis data. All cell intensity data associated with the probe analysis data.

To select the tasks, use either of the following methods:

Using the Drag-and-Drop Method

1. Drag the task item that you want to publish from the data tree onto a database icon in the top pane of the publish window.
2. Repeat the drag-and-drop operation for the remaining task items to be published **(Figure 12.5)**.

Using the Add Toolbar Button

1. Click the **Add** button or select **Publish** →**Add Item** from the menu bar.

   ⇒ The Open dialog box appears **(Figure 12.3)**.

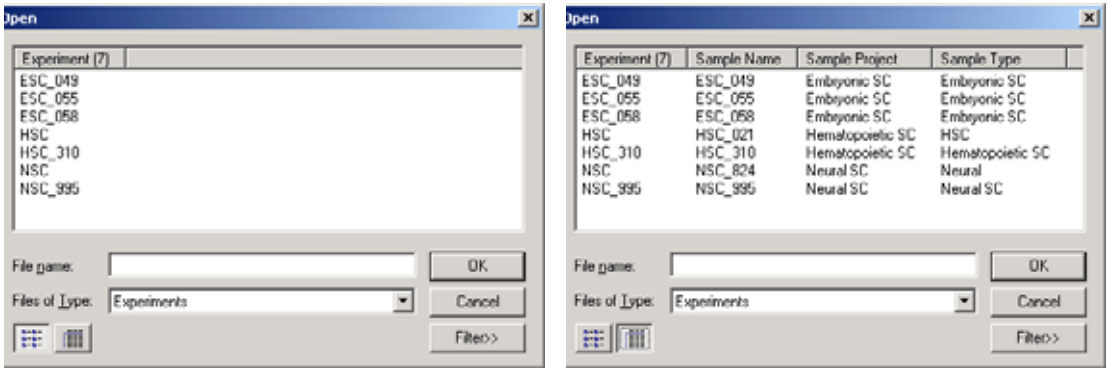


Figure 12.3
Open dialog box, list view (left) and details view (right)

2. Click **Details** to display experiment and sample information for each data type.
3. To filter the list of data types in the Open dialog box, click **Filter**
   ⇒ The advanced filters are displayed **(Figure 12.4)**.

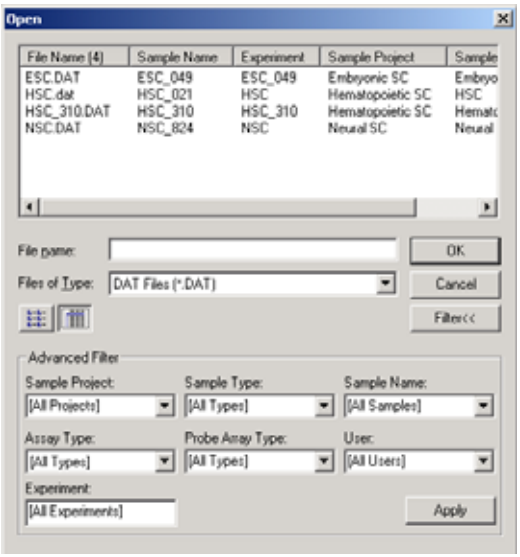


Figure 12.4
Open dialog box, advanced filter

4. Make selections from the drop-down lists and click **Apply** to filter the list.

5. Make a selection from the **Data Type** drop-down list and select the data to be published.

   To select adjacent data, press and hold the **Shift** key while you click the first and last name in the selection. To select non-adjacent data, press and hold the **Ctrl** key while you click the names.

6. Click **OK** to add the data to the selected publish database **(Figure 12.5)**.

7. To remove a task item from the publish window, select the task item in the publish window and click the **Remove** button ✕. Alternatively, choose **Publish →Remove Item** from the menu bar.

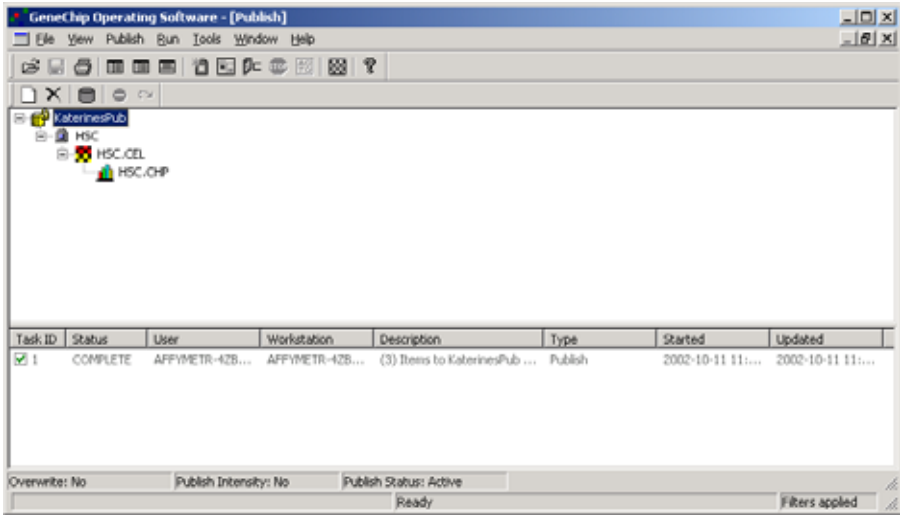


Figure 12.5
Publish window

Publishing Options

You may publish intensity data or overwrite a previously published task item. You must be authorized by the GCOS administrator to overwrite data. The status bar at the bottom of the publish window displays the publishing options **(Figure 12.5)**.

Publishing Intensity Data

Cell intensity data (.cel) are not automatically published. Publishing .cel data uses large amounts of computer memory. For example, publishing the probe analysis data for a high density probe array requires approximately 2 MB of disk space compared to 30 MB for both the probe analysis and cell intensity data.

✔ Note *The image data are not published.*

1. To include cell intensity data in the publish database, select **Publish** → **Options** →**Publish Intensities** from the menu bar.

   ⇒ The **Publish Intensities** menu item is check marked.

2. To not include cell intensity data in the publish database, select **Publish** →**Options** →**Publish Intensities** from the menu bar to remove the check mark.

Overwriting Data

A previously published task item can be overwritten. Users must be authorized by the GCOS administrator to overwrite data.

- Select **Publish** →**Options** →**Overwrite Data** from the menu bar.

Publishing, Canceling, or Restarting a Task

Note

*It is recommended that users **not publish and restore** to the same publish database simultaneously. Sometimes the restore will be rejected and sometimes the publishing will be rejected. If the restore of a publish database fails, an error message is displayed.*

Publishing the Task

1. After you have specified the task, click the Publish button . Alternatively, select **Publish →Publish** from the menu bar.
   ⇒ The Publish Login dialog box appears if this is the first time during the session that an item has been published to the database **(Figure 12.6)**.

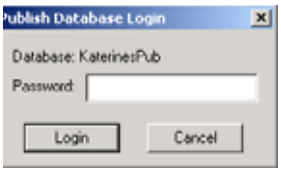


Figure 12.6
Publish Database Login

2. Enter the password for the database.
   ⇒ The task is displayed in the task log and will be published during the time frame specified by the GCOS administrator.

Canceling a Task

You can cancel tasks waiting to be published (the status in the task log is WAIT).

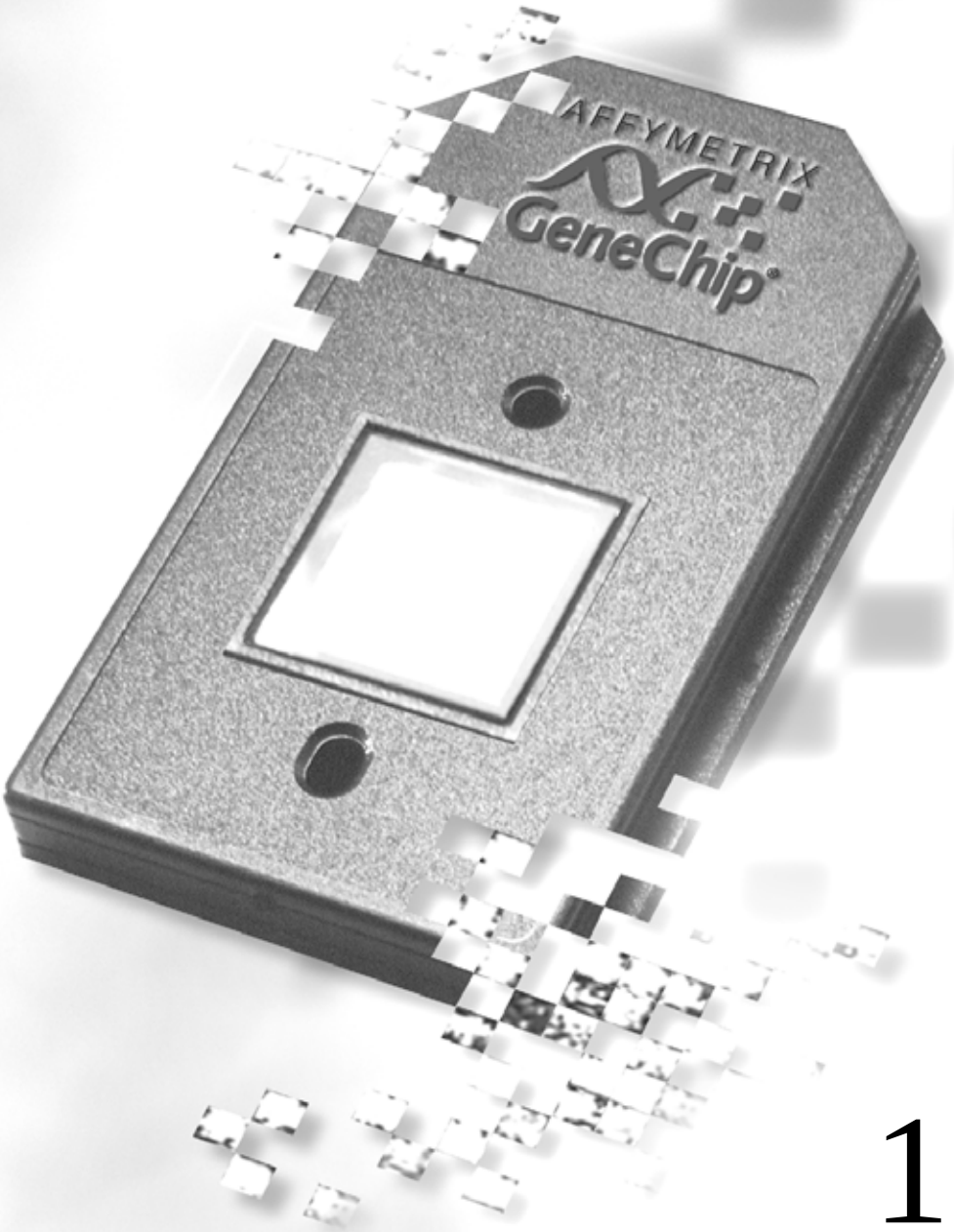
1. Select the task in the task log.
2. Click the **Cancel** button . Alternatively, select **Publish →Monitor → Cancel Publish** from the menu bar.
   ⇒ The task is now canceled (the status is CANCELED).

Restarting a Publishing Task

You can reinstate or *restart* a canceled task and return it to the publishing queue.

1. Select the canceled task in the task log.
2. Click the **Restart** button . Alternatively, select **Publish** →**Monitor** → **Restart Publish** from the menu bar.

   The task is now restarted (the status is WAIT) and will be published during the time frame specified by the GCOS administrator.



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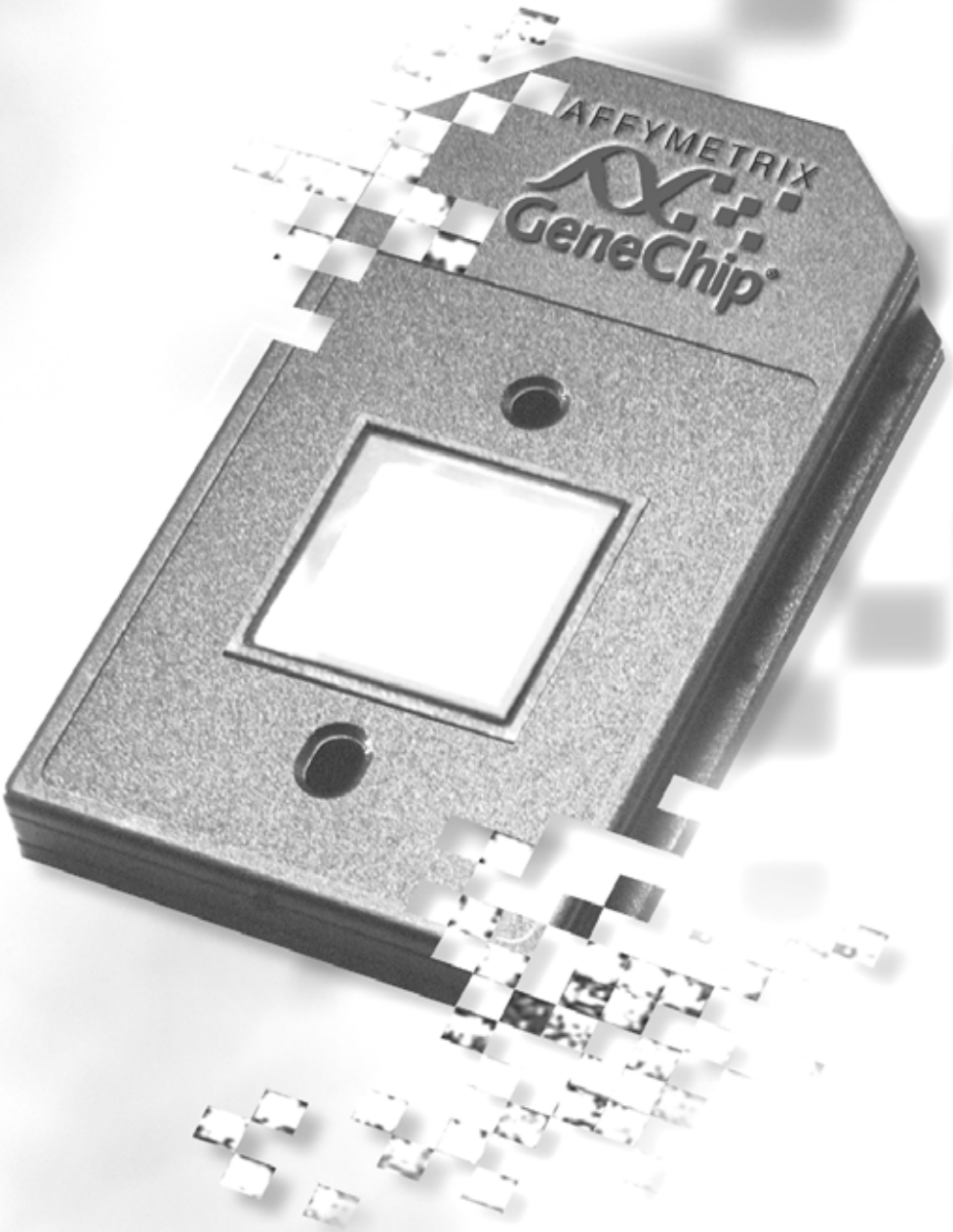


Defaults

This chapter explains how to change the factory set defaults for:

- image settings
- data locations
- fluidics station configuration
- analysis prompts
- experiment data storage

Viewing Default Settings

- Select **Tools** →**Defaults** from the menu bar.

  ⇒ The Defaults dialog box appears **(Figure 13.1)**.

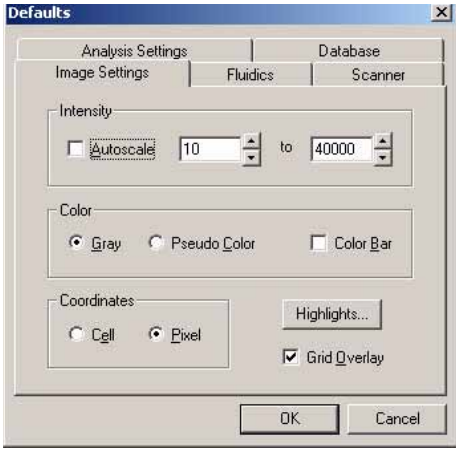


Figure 13.1
Defaults dialog box, Image Settings tab

Image Settings

The image settings **(Figure 13.1)** affect the display of image and cell intensity data in the Image window. For more information on this window, see *The Image Window* on page 135.

✔ Note

The image settings specified in the Defaults dialog box (except for the color option) are applied to subsequently opened image or cell intensity data and do not affect active, open data.

Intensity Range

GCOS software records a dynamic intensity range from 0 to 65,000 and provides 256 colors from black through white for image display. The intensity range of an image is divided into 256 bins and each bin is assigned a color or gray scale.

You may enter a new lower or upper limit for the intensity range associated with an image and apply this subset of the dynamic range to the image. Lowering the upper limit increases the image brightness and raising the lower limit decreases the brightness.

Using this *image scaling* process, you can adjust the display of a data or cell intensity file for optimum viewing. Alternatively, GCOS can automatically scale the image using the minimum and maximum pixel intensities of the image.

1. To automatically scale an image, choose the **Autoscale** option in the Defaults dialog box **(Figure 13.1)** and click **OK**.
2. In the **Image Settings** dialog box for the current image or cell intensity data, you may click **Defaults** to return the intensity parameters (Autoscale option and Intensity range) to the settings in the Image Settings tab of the **Defaults** dialog box. See *Making Adjustments to the Current Image or Cell Intensity Data Only* on page 144 for more information about the Image Settings dialog box.

Color

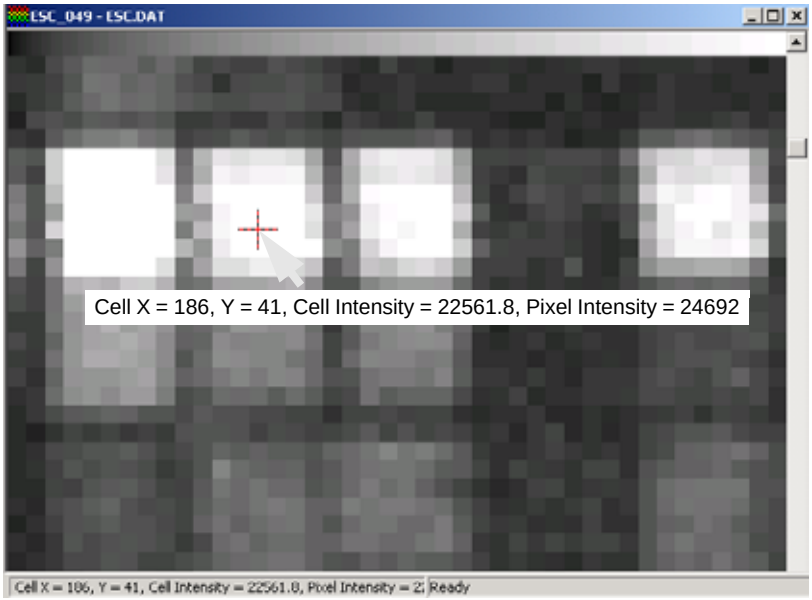
You can view the pixel intensity values in gray scale or pseudo color (generated by applying rainbow colors to the intensity scale). Choose the **Color Bar** option **(Figure 13.1)** to display a gray scale or pseudo color bar at the top of the Image window.

Coordinates

When you click the image, a red cross hairs marks the location. The status bar at the lower left of the main window displays the intensity data and x-y coordinates of a probe cell or pixel indicated by the cross hairs.

If you choose the **Cell** option in the Defaults dialog box **(Figure 13.1)**, a pop-up tool tip and the status bar display the x-y coordinates of the cell, the cell intensity, and the pixel intensity **(Figure 13.2)**.

If you choose the **Pixel** option, the pop-up tool tip and the status bar display the x-y coordinates of the pixel and the pixel intensity **(Figure 13.2)**.



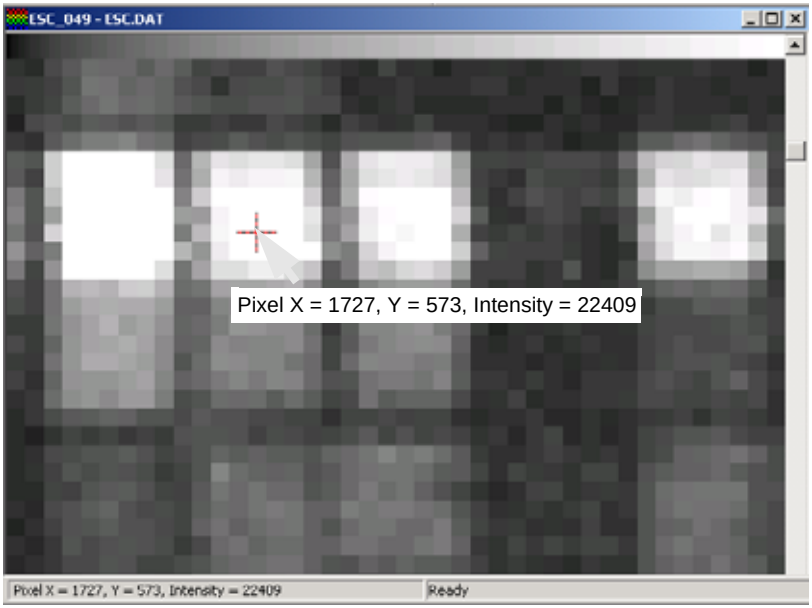


Figure 13.2
Image data, probe cell coordinates (top) and pixel coordinates (bottom)

Highlight Colors

You may specify new highlighting colors for the grid, masked cells, outlier cells, or highlighted probe array cells (features).

1. Click **Highlights** in the Defaults dialog box **(Figure 13.1)**.

   ⇒ The Highlight Colors dialog box appears **(Figure 13.3)**.

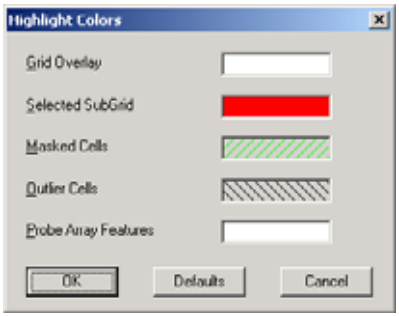


Figure 13.3
Highlight Colors dialog box

2. To change the color of a particular item (for example, the grid overlay):

   a. Click the associated color box in the Highlight Colors dialog box.

   ⇒ The Color palette is displayed **(Figure 13.4)**.

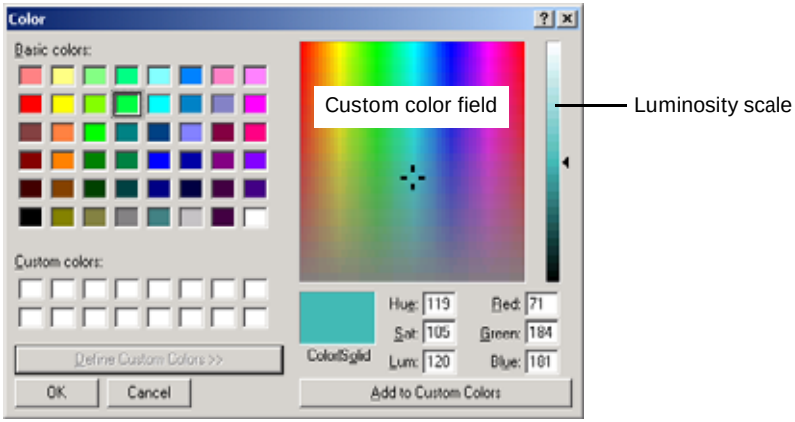


Figure 13.4
Color palette

b. To select a predefined color, click one of the basic colors.

c. To define a custom color, click **Define Custom Colors**, then use the click-and-drag method to move the cross hairs in the custom color field. Adjust the color brightness using the luminosity scale to the right. When finished, click **Add to Custom Colors** to apply the color.

   ⇒ The new color is applied to the selected item.

d. Click **OK** to close the Color palette.

Grid Overlay

Choose the **Grid Overlay** option **(Figure 13.1)** to automatically display the grid on a image or cell intensity data. Remove the check mark to toggle the grid overlay off. Alternatively, click **Grid** in the Image window task bar (or press the **G** key) to toggle the grid on or off.

Fluidics Settings

In the Fluidics tab **(Figure 13.5)** you can:

- specify the number of fluidics stations installed on the system (up to eight fluidics stations may be simultaneously controlled by one work station)
- specify each fluidics station's number and ID

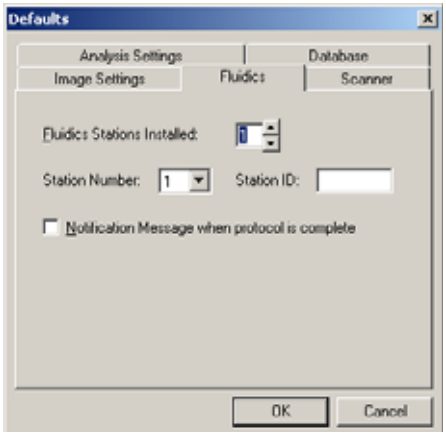


Figure 13.5
Defaults, Fluidics tab

Notification Message when protocol is complete Choose this option to display a notification of completion at the end of a protocol

Scanner Settings

In the Scanner tab **(Figure 13.6)** you can:

- specify whether a scanner is installed on the system
- if a scanner is installed, choose the option to turn on the laser at startup
- if the AutoLoader is installed, choose to use it in manual mode
- if the AutoLoader is installed, choose to disable it.

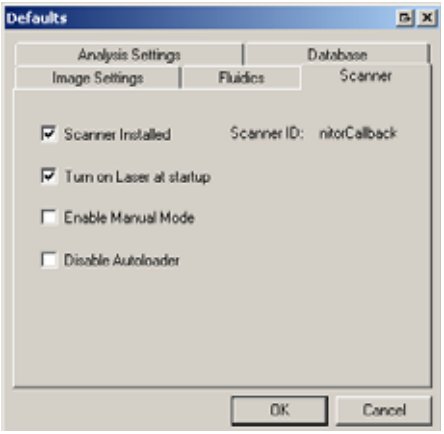


Figure 13.6
Defaults, Scanner tab

Scanner Installed	Choose this option after the scanner is installed.
Turn on Laser at startup	Confirm this option is chosen (default) so that the laser automatically turns on when GCOS is started.
Enable Manual Mode	Choose this option if you are using a scanner equipped with the AutoLoader and would like to disable the AutoLoader's autorun feature. This will allow you to add and scan cartridges one at a time.
Disable AutoLoader	Choose this option if you are using a scanner equipped with the AutoLoader and would like to disable the AutoLoader completely. This will allow you to use the software without communicating with the AutoLoader.

Analysis Settings

The Analysis Settings tab **(Figure 13.7)** displays two options that are available when an analysis is run.

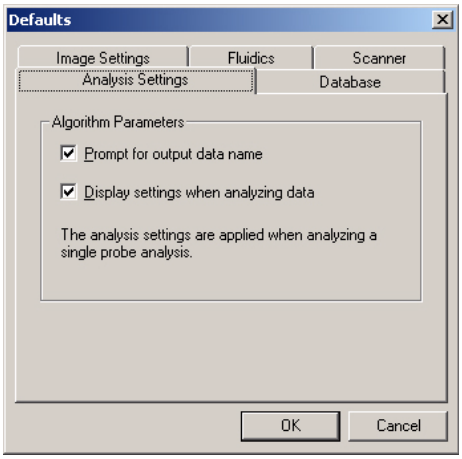


Figure 13.7
Defaults, Analysis Settings tab

Prompt for Output Data Name

1. Choose the **Prompt for output data name** option **(Figure 13.7)** to display a Save Results As dialog box **(Figure 13.8)** at the start of an analysis.

   The default name for the probe analysis data is the same as the experiment name. The Save Results As dialog box displays the default name.

Figure 13.8
Save Results As dialog box

2. Click **OK** to keep the default data name. Alternatively, enter a new name and click **OK**.

Display Settings When Analyzing Data

Choose the **Display settings when analyzing data** option **(Figure 13.7)** to display the Expression Analysis Settings dialog box **(Figure 13.9)** at the start of an analysis.

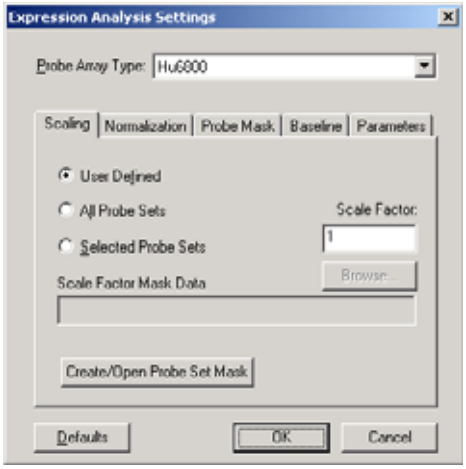


Figure 13.9
Expression Analysis Settings dialog box

Database

The Database tab **(Figure 13.10)** specifies how GCOS manages experiment data, including image, cell intensity, and probe analysis data.

Choose the **GCOS Server** option to store the experiment data on the remote GCOS server. If this option is not chosen, the experiment data are stored on the MSDE database on the workstation.

Note *The Experiment Data Storage option is only available if all windows are closed and no instruments are active.*

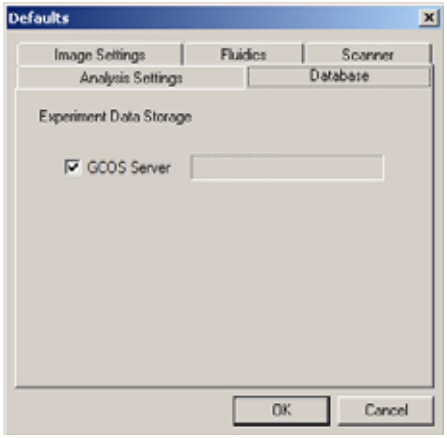
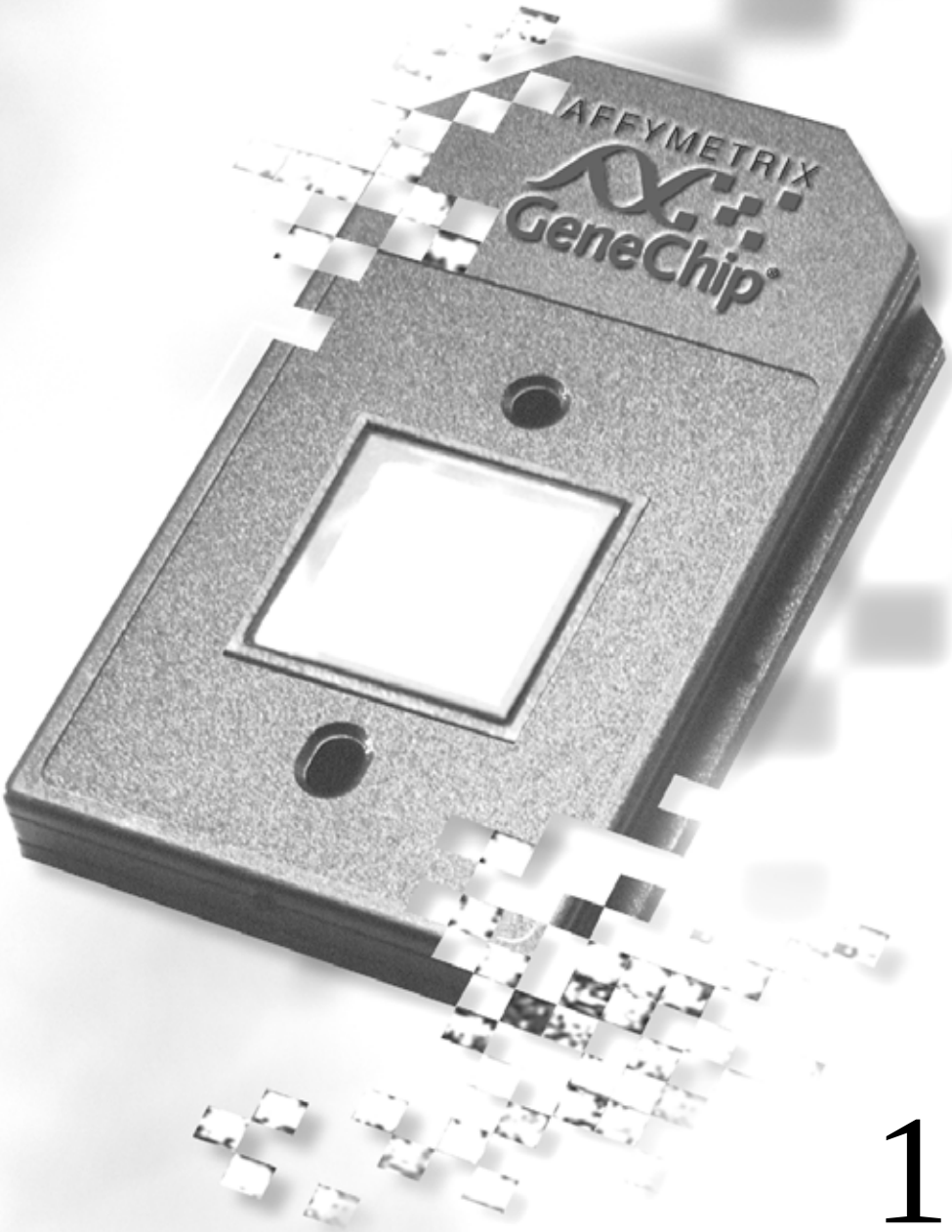


Figure 13.10
Defaults, Database tab



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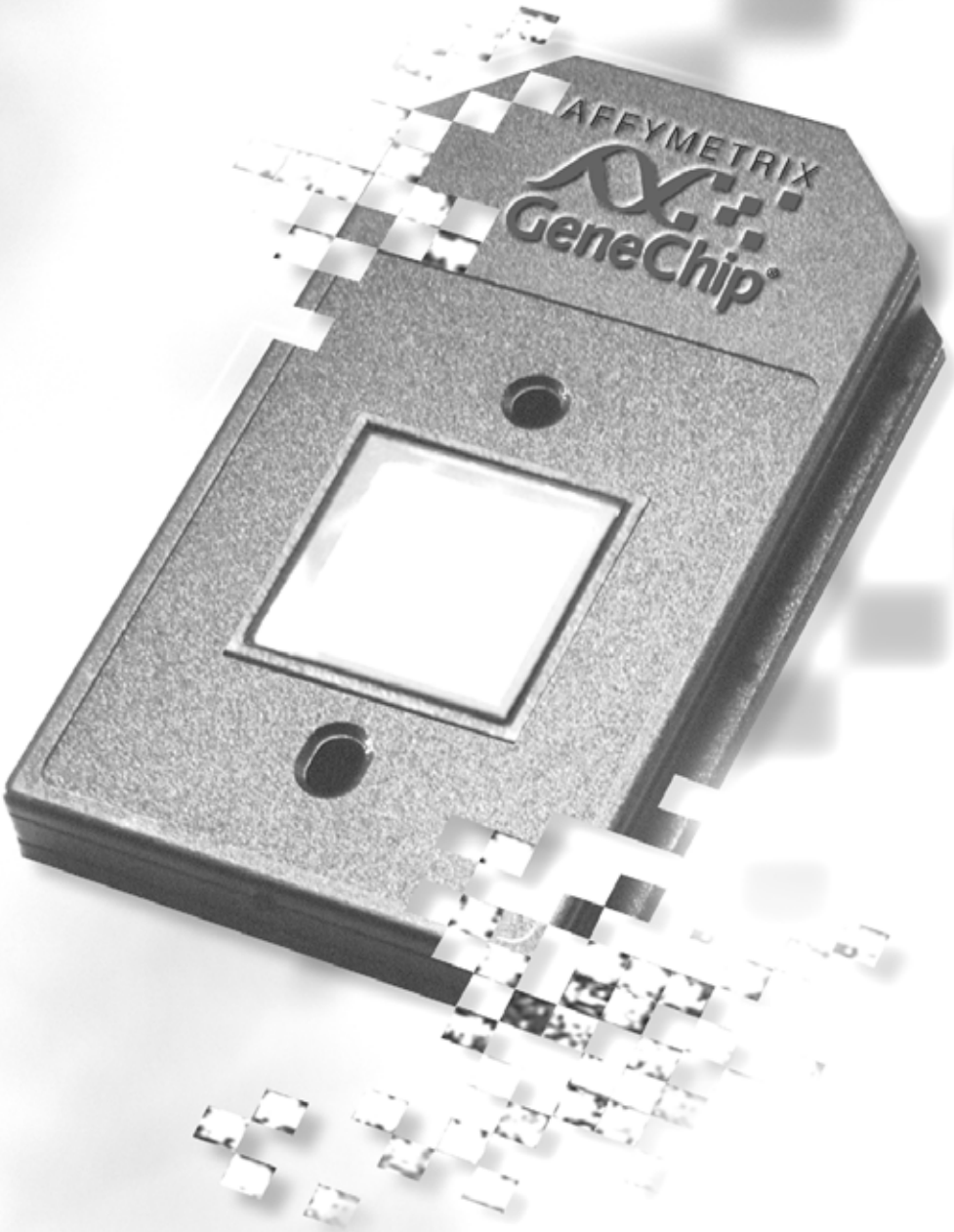


Printing

This chapter explains how to print:

- image data
- cell intensity data
- probe analysis data
- reports

Printing Image or Cell Intensity Data

1. When the Image window is open, click the **Print** button or select **File →Print** from the menu bar.

   ⇒ The Print dialog box appears **(Figure 14.1)**.

   No print range options are available.

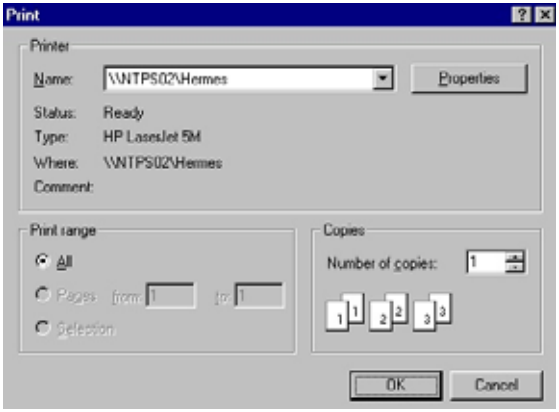


Figure 14.1
Print dialog box in the Image window

2. Click **OK** to print the image or cell intensity data.

Printing Probe Analysis Data

You may print tabular data and graphs from the Expression Analysis window (EAW).

1. When the EAW is open, click the **Print** button or select **File →Print** from the menu bar.

   ⇒ The Print dialog box appears **(Figure 14.2)**.

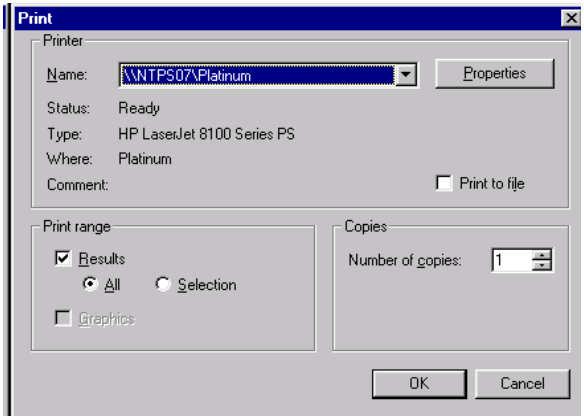


Figure 14.2
Print dialog box, EAW

The **All** and **Selection** options apply only to the Experiment Information, Metrics, and Pivot tables. If the **Graphics** option is chosen, the displayed graphs are automatically printed.

2. Choose **Results** and:
   - **All** to print the entire table in a tab (Experiment Information, Metrics, or Pivot).
   - **Selection** to print only highlighted table rows.

Printing an Expression Report

You may print all or selected pages from an expression report.

1. When a report is open in the main display area, click the **Print** button or select **File →Print** from the menu bar.

   ⇒ The Print dialog box appears **(Figure 14.3).**

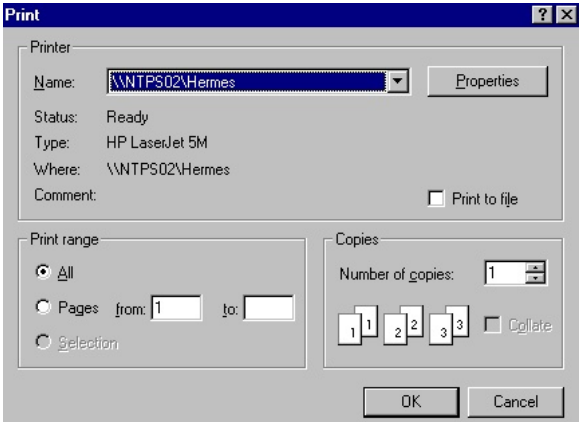
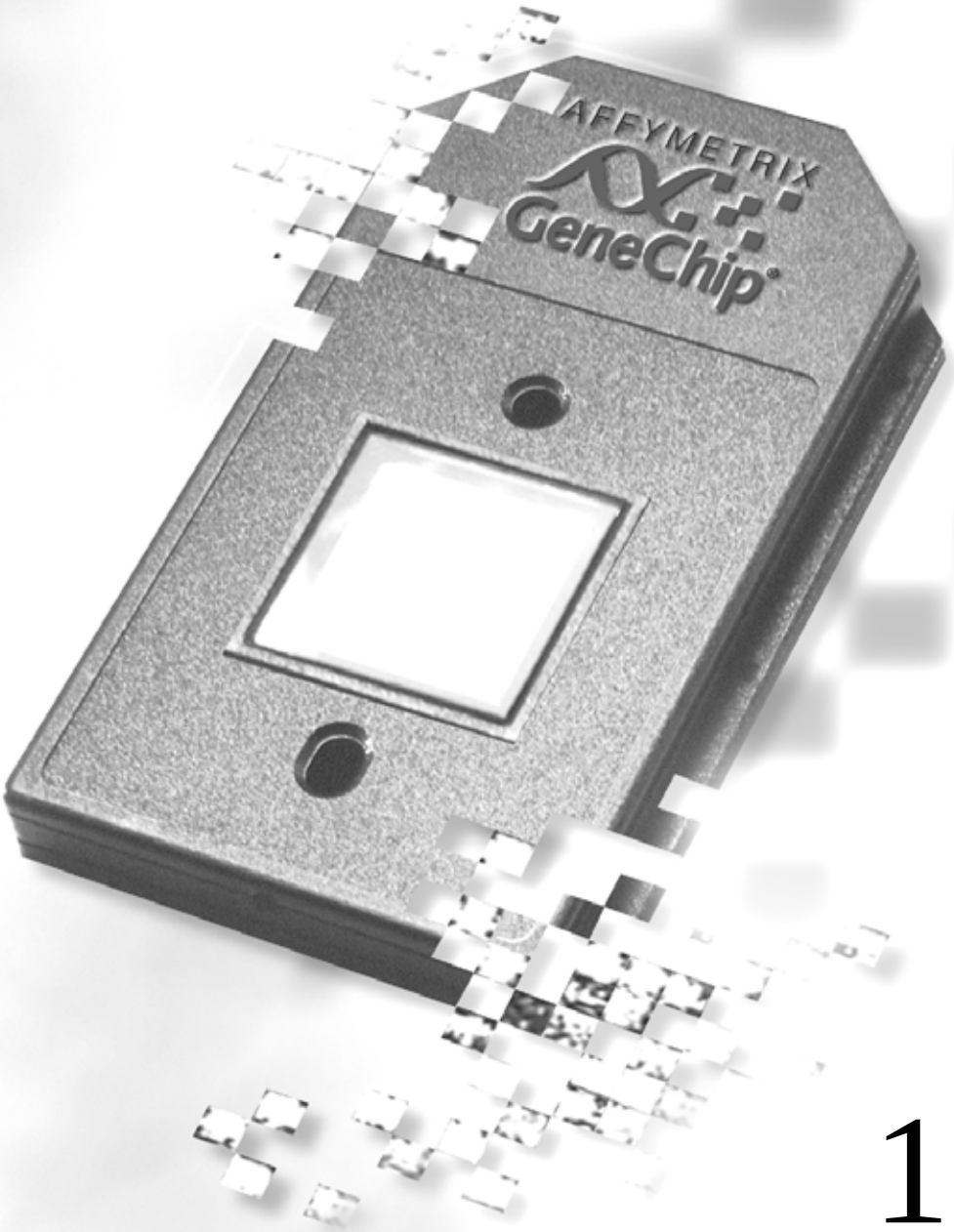


Figure 14.3
Print dialog box, report (.rpt)

2. Choose:
   - **All** to print all pages of a report.
   - **Pages** and enter a page range to print selected pages of a report.



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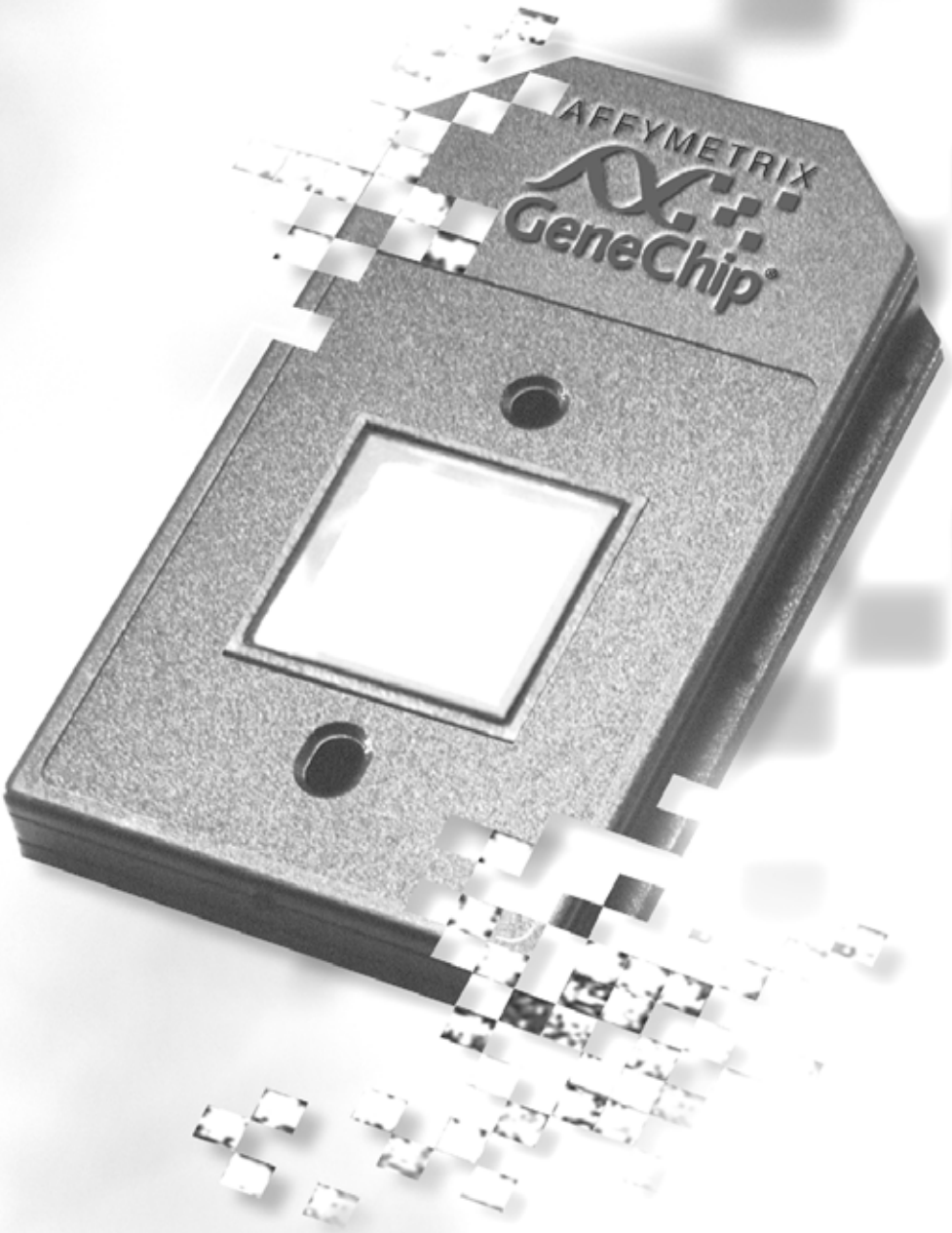


GCOS Manager

The GCOS Manager application provides software tools to:

- manage the GeneChip® data in the process and publish databases
- define and manage templates for sample registration and experiment setup
- create usersets for analysis
- create and manage publish databases
- import data to the process database
- export experiment and analysis data
- archive and restore analysis data
- create and manage roles that define user access privileges (available only to users connected to a GCOS server)

This chapter explains the GCOS Manager functions and how to use them.

Getting Started

1. To start the GCOS Manager, click the Windows **Start** menu button Start, then select **Programs →Affymetrix →GCOS Manager**.

   ⇒ The GCOS Manager starts and displays the user interface **(Figure 15.1)**.

   The software functions are organized in the following tabs: Process, Publish, Import, Userset, Template, and Roles.

Note *The Roles tab is available when the GCOS Manager is connected to a GCOS server.*

   Table 15.1 lists the functions found in each tab.

2. To select the active server, make a selection from the server drop-down list in the toolbar **(Figure 15.1)**.

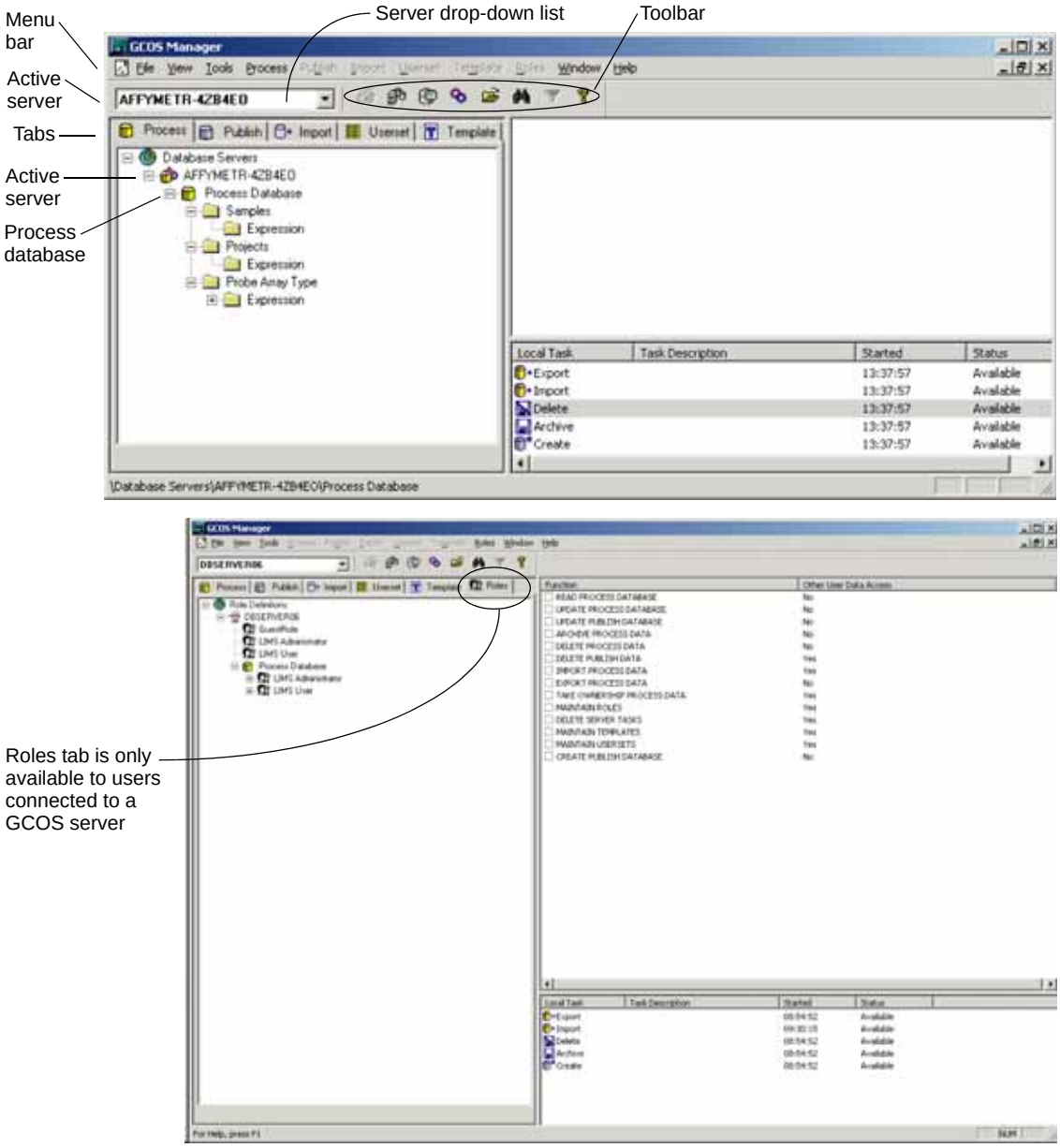


Figure 15.1
GCOS Manager user interface, Microsoft® MSDE workstation database (top), GCOS server database (bottom)

Table 15.1
GCOS Manager tab functions for Affymetrix® GeneChip® data

GCOS Manager Tab	Functions in This Tab
Process	Archive or restore experiment data (.dat, .cel, .chp). Delete process data. Export experiment data. Assume ownership of data (available only to users connected to a GCOS server). Print an experiment. Register or unregister the local workstation or GCOS server.
Publish	Delete published data or a probe array type from a publish database. Create a publish database.
Import	Import experiment data to the process database
Userset tab	Create or modify a userset (a set of predefined parameter settings for the Statistical Expression algorithm). Delete a probe array type from a userset. Delete a userset.
Template	Create a template (a predefined form used to register samples and define experiments in GCOS 1.0). Deactivate or activate a template for use in GCOS 1.0. Rename a template.
Roles (available only to users connected to a GCOS server)	Create a role (a defined set of user privileges). Delete a role.

GCOS Manager Icons

Table 15.2 shows the icons used in GCOS Manager.

Table 15.2
GCOS Manager Icons

	Sample		Template		Properties information
	Project		Global		Gray bullet indicates processes that have been completed.
	Experiment information		Server		Green bullet indicates processes waiting to commence.
	Scanned probe array image data (.dat)		Process database (yellow)		Red check mark on an experiment, .dat, .cel, or .chp icon(s) indicates that the data already exist in the publish database.
	Computed cell intensities (.cel)		Publish database (gray)		Export status
	Probe analysis data (.chp)		Import to database		Import status
	Probe array type		Domain		Delete status
	Chip		User group		Archive status
	Vessel		User		Create publish database status
	Userset		Role		Filter

Terminology

Table 15.3 lists terms that are used frequently throughout this chapter. For your convenience, the terms are listed under the tabs where they are most frequently found.

Table 15.3
Terminology common to all tabs

Data & Object Types Across All Tabs	Definition
Automate	A monitored task whose status, either on or off, is viewed in the **Local Task** pane. **Automate On** indicates GCOS will automatically track an experiment through data analysis after the probe array has been scanned.
Sample	A set of experiments.
Experiment	Specifies the type of probe array to which the target (sample) will be hybridized as well as other information relevant to the experiment. The experiment name provides the name for the data types that are subsequently generated in the analysis. Note: The Import tab shows experiment, cell intensity, or probe analysis data that can be imported or copied to the process database. In GCOS, the process database includes the experiment and sample data.
Image data (.dat)	The .dat file contains the image data of the scanned probe array data acquired in GCOS. The image data along with the image attributes stored in the database define the raw image data.
Cell intensity data (.cel)	The cell file (.cel) of computed intensities that GCOS automatically generates from the image data file (.dat). GCOS can publish the .cel to a publish database where it is accessible to the Affymetrix® Data Mining Tool (DMT).
Probe analysis data (.chp)	The probe analysis output or chip file that is generated by the GeneChip data algorithms. GCOS can publish expression .chp to a publish database where it is accessible to the Affymetrix® DMT.

Table 15.4
Terminology in the Process tab

Process Tab Terms	Definition
Active server	More than one GCOS server may be registered. The active server refers to the GCOS server where the displayed process database resides.
Archive	Move files from the process database to an external location.
Unarchive	Restore archived files to the process database.
Export	Copy data from the process database and send to an external location. Note: For an export from GCOS to Microarray Suite (MAS) 5.x, data created in GCOS are used to generate data files that are added to the default file location in MAS.
Process database	Database residing on the workstation or GCOS server that contains experiment and sample data. There is only one process database per workstation or GCOS server.
Project	A set of experiments with a common bond that are grouped together by their affiliation with a project. A project is created during sample registration in GCOS.

Table 15.5
Terminology in the Publish tab

Publish Tab Terms	Definition
Affymetrix® Analysis Data Model (AADM)	The AADM is the relational database schema that Affymetrix uses to store experiment data (.dat, .cel, .chp) in the publish database so that results may accessed by the Affymetrix® Data Mining Tool and other third party analysis tools. The AADM includes additional tables to support expression results. The AADM is publicly available to support open access to experiment information generated and managed by Affymetrix® software.
Cell intensities	The computed intensities that GCOS automatically generates from the image data (.dat). GCOS can copy or *publish* the cell intensity data to a publish database.
Publish database	An AADM database residing on a server that contains select published process data. More than one publish database may reside on a single server.
Publish	To copy or *migrate* experiment data to an AADM database on the workstation or GCOS server.

Table 15.6
Terminology in the Import tab

Import Tab Terms	Definition
Create Sample	Creating a sample captures sample information and identifies the relationship between the experiment and experiment data (.cel, .chp) that are being imported.
Import	Copies experiment data (.cel, .chp) created in GCOS to the process database on the workstation or the GCOS server. Note: For an import to GCOS from Microarray Suite (MAS) 5.X, data are extracted from the experiment data files created in MAS and are added to the process data base.

Table 15.7
Terminology in the Userset tab

Userset Tab Terms	Definition
Userset	A predefined set of values for modifiable parameters in the Statistical Expression algorithm.
Independent parameter	Static settings defined in the userset that are not influenced by the probe array type.
Dependent parameters	Settings defined in the userset that are specific to a particular probe array type.

Table 15.8
Terminology in the Template tab

Template Tab Terms	Definition
Activate	Restores an inactive (deactivated) attribute or template.
Template	A form created by the administrator that defines the attributes and values which are required to register samples and define experiments in GCOS.
Deactivate attribute	Renders an attribute inactive. The attribute is not permanently removed from the system. A deactivated attribute does not appear in GCOS until it is restored by the **Activate** command.
Deactivate template	Permanently deletes a template from the system if it is not currently used by an experiment or sample. Otherwise, the template is deactivated and does not appear in GCOS until it is restored by the **Activate** command.

Table 15.9
Terminology in the Roles tab

Roles Tab Terms	Definition
Privileges	The functions or permissions that define a role.
Role Definitions	Identifies the first branch under the active server where the role names are listed.
Role	A defined set of user access privileges.
User Definitions	Identifies the branch under the process database where role membership is defined.

Process Tab

This section of the manual explains the layout of the Process Tab window, its functions, and how to accomplish tasks here.

The Process tab shows you the data in the system for all registered servers, where the data are found, and environmental property information about the data.

The Process tab window is divided into three windowpanes **(Figure 15.2)**.

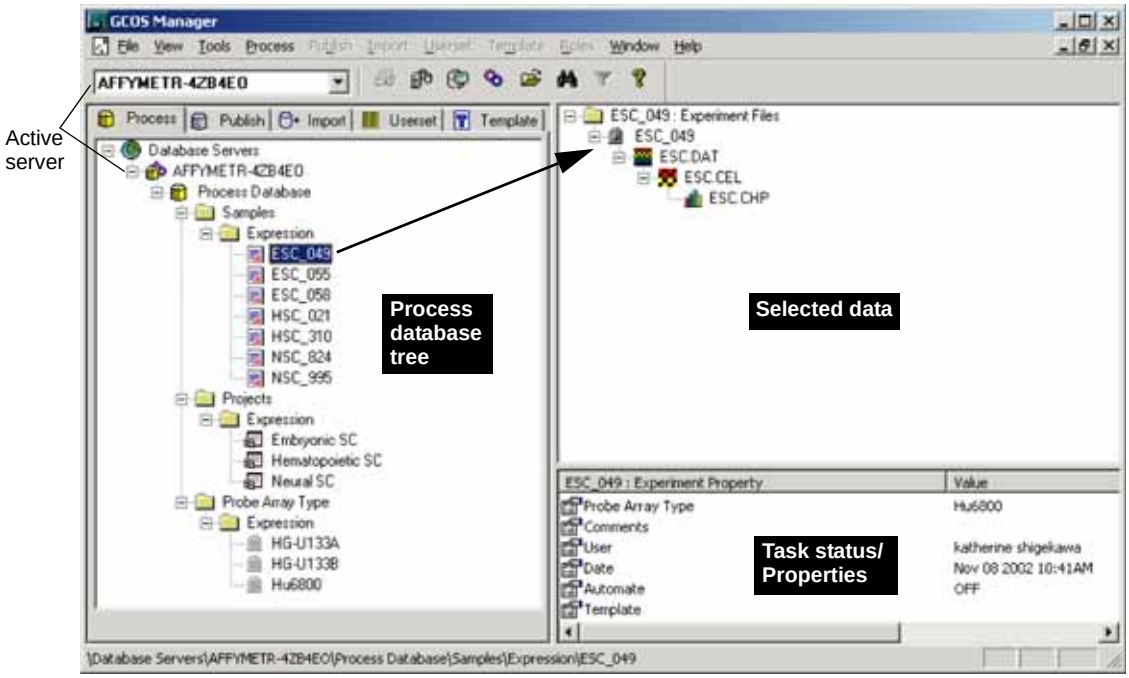


Figure 15.2
Process tab windowpanes

Process Tab Windowpanes

Process database tree	Shows the active server, process database, and data types (samples, projects, and types of probe arrays). The data type subfolders contain samples, projects, or probe array types. Click a sample or project to display the associated experiment data (.dat, .cel, .chp) in the data pane.
Selected data	Shows the experiment data of the sample or project selected in the process database tree. The first time you click an experiment in the tree of selected data, the information is read and a + sign appears next to the experiment name. Click the + sign to display the associated data.
Task status/ Properties	Displays the property information or local task view, depending on the item selected in process database tree or the selected data pane. Property information is defined by the template that was used during sample registration in GCOS. The task status view shows the status of processes that may be occurring in the database (for example, export, import, delete, archive, or create). In the task status view, you can monitor these jobs while you work on other tasks in the system.

Process Tab Functions

The primary tasks in the Process tab are:

- archive or unarchive data
- delete data
- export data
- assume ownership of data
- print data

The primary data types in the Process tab are:

- sample
- project
- experiment
- image data, cell intensity data, and probe analysis data

The tasks associated with each data type are found under **Process** in the menu bar. Alternatively, right-click an item to view a shortcut menu **(Figure 15.3)**.

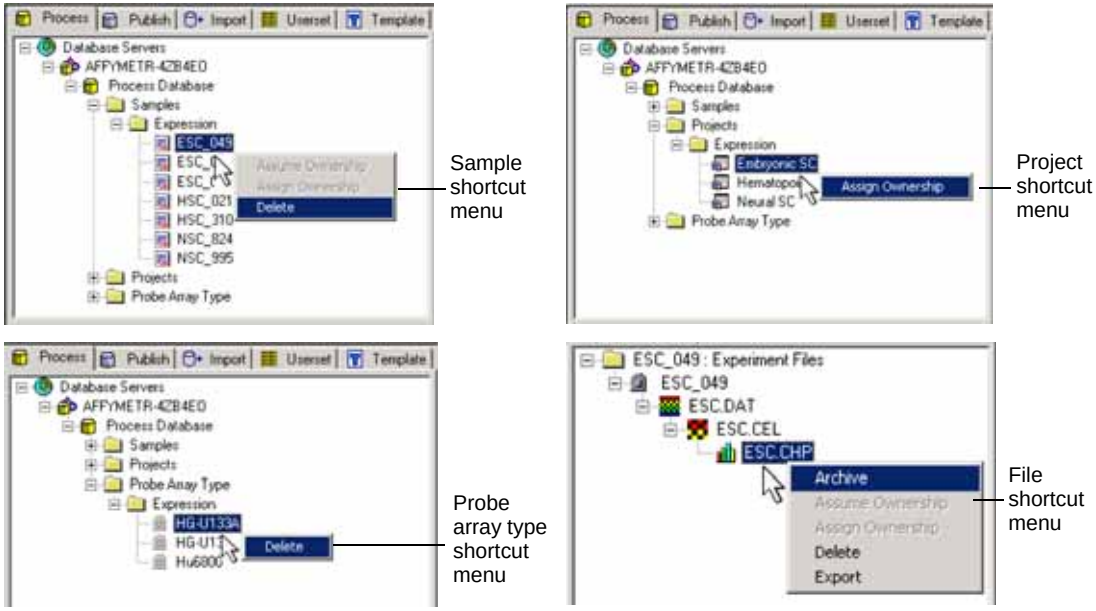


Figure 15.3
Process tab shortcut menus

Archiving Data

Archiving data moves a user-selected data type to an external storage location. An archived data type no longer exists in the process database; however, its representative icon remains in the data tree and appears gray to indicate the data are available through the unarchive process.

1. In the Process tab, click the sample or project that contains the data you want to archive.
2. In the data pane, right-click the data that you want to archive and select **Archive** from the shortcut menu that appears **(Figure 15.3)**. Alternatively, click the data and select **Process** →**Archive Data Path** from the menu bar.

   ⇒ The Select Archive Data Path dialog box appears **(Figure 15.4)**.

Figure 15.4
Select Archive Data Path dialog box

3. Select a drive from the **Drive** drop-down list and navigate to the directory where you want to archive the data.
4. Click **OK**.

   ⇒ The data are archived in the specified directory.

✔ Note

To view more information about the archive task status, right-click Archive *in the Task Status/Properties pane and select* ***View Task Log*** *from the shortcut menu that appears* **(Figure 15.5)**. *Alternatively, double-click* Archive to view the archive task log **(Figure 15.6)**.

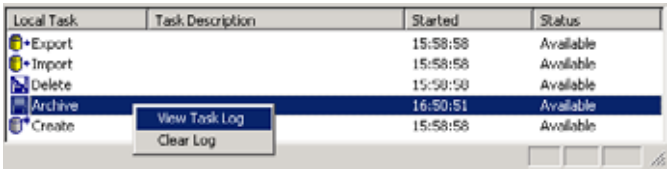


Figure 15.5
Task Status/Properties pane

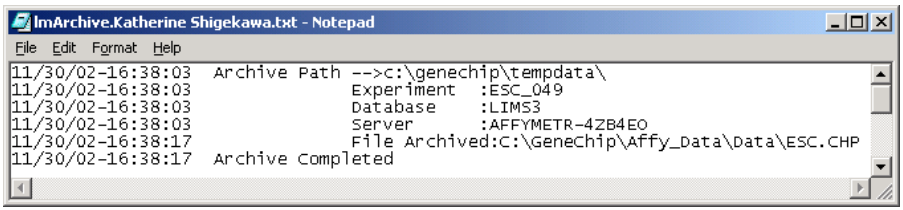


Figure 15.6
Archive task log

Note

*Use the **Find** function to generate a list of files associated with a specific project. Next, SHIFT-select and archive the data in one quick step. This is only available for .dat, .cel, and .chp files in the process database.*

Unarchiving Data

The icon for archived data appears gray. The unarchive command restores archived data to the process database.

1. In the Process tab, click the sample or project that contains the file you want to unarchive.
2. In the data pane, right-click the data that you want to unarchive and select **Unarchive** from the shortcut menu that appears (**Figure 15.3** on page 285). Alternatively, click the data and select **Process →Unarchive** from the menu bar.

   Selecting the parent data includes all related child data in the unarchive process.

   If GCOS Manager cannot find the archived file, a Data Location dialog box appears. Enter the location of the data you want to unarchive in this dialog box.

   ⇒ The data are unarchived and restored to the process database on the active server.

Note

*The Process window does not automatically refresh upon completion of the **Archive** or **Unarchive** task. Therefore, if you unarchive data, the icon remains gray even though the task was completed successfully. To refresh the view, select **View →Refresh** from the menu bar.*

Changing Data Ownership

If needed for security or management purposes, you can assume or assign ownership of data (change the user attribute of data).

Note *This feature is only available to users connected to a GCOS server.*

If the **Take Ownership Process Data** option is defined for a user in the Roles tab, the user can:

- assume ownership of samples, experiments, or analyses
- assign ownership of samples, experiments, or analyses to a user who is also a member of a user group that the assignor belongs to

(For more information on role definition, see *Roles Tab*, on page 357).

Data ownership means you have permission to overwrite a file with new contents. For example, an owner can reanalyze cell intensity data using different parameter settings for the Statistical Expression algorithm to generate new probe analysis data.

Assuming Ownership

1. In the Process tab, right-click the sample, project, or data of interest.
2. Select **Assume Ownership** from the shortcut menu that appears.
   ⇒ Your user name is displayed in the Task Status/Properties pane **(Figure 15.8)**.

Assigning Ownership

1. In the Process tab, right-click the sample, project, or data of interest.
2. Select **Assign Ownership** from the shortcut menu that appears.
   ⇒ The Assign Owner dialog box appears **(Figure 15.7)**.

Figure 15.7
Assign Owner dialog box

3. Enter the name of the new owner, and click **OK**.

   ⇒ The user name of the new owner is displayed in the Task Status/ Properties pane **(Figure 15.8)**.

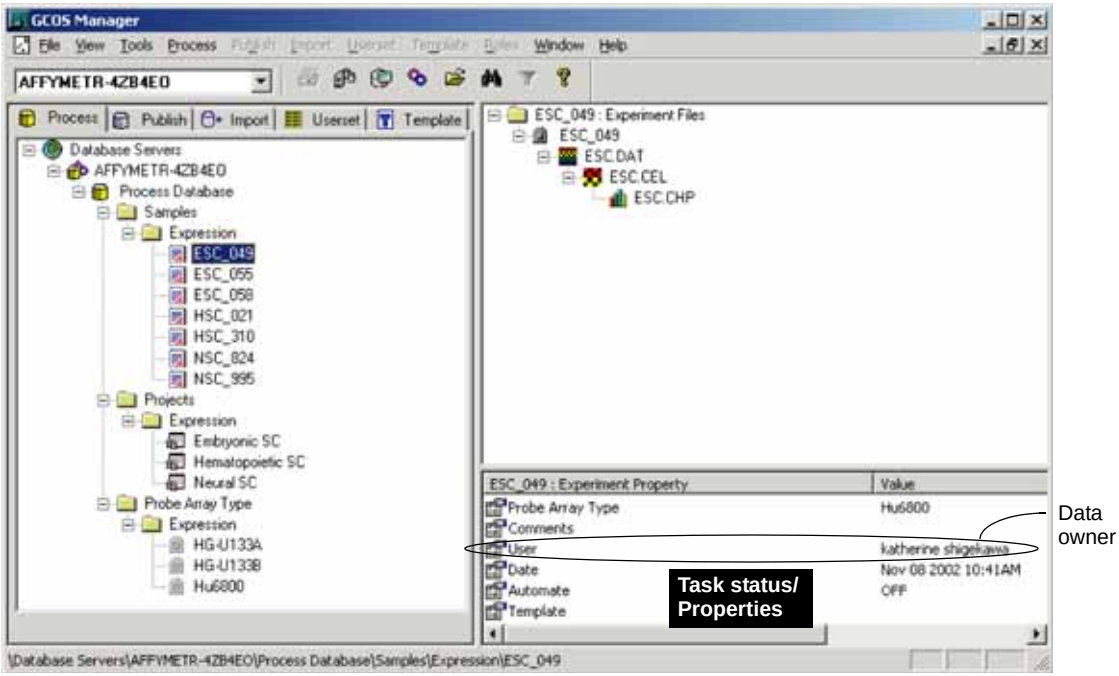


Figure 15.8
Process tab, Task status/Properties pane shows the user name of the owner of the selected data

Deleting Process Data

You can delete samples, experiments, experiment data (.cel, .chp), or usersets. Deleting a sample deletes all associated experiments and analyses (.cel, .chp). Deleting an experiment deletes the associated analyses (.cel, .chp).

! CAUTION

*The **Delete** option permanently removes data from the database. There is no restore function to recover deleted data.*

1. Right-click the item that you want to delete, and select **Delete** from the shortcut menu that appears. Alternatively, click the item and select **Process →Delete** from the menu bar.

   ⇒ A warning/confirmation box appears **(Figure 15.9)**.

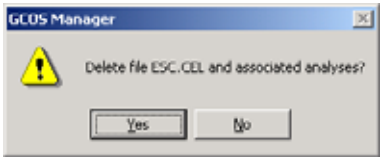


Figure 15.9
Delete warning/confirmation box

2. Click **Yes** to continue.

   ⇒ The Task Status/Properties pane displays the delete local task status.

✔ Note

To view more information about the delete task status, right-click Delete *in the Task Status/Properties pane and select **View Task Log** from the shortcut menu that appears* **(Figure 15.10)**. *Alternatively, double-click* Delete *to view the delete task log* **(Figure 15.11)**.

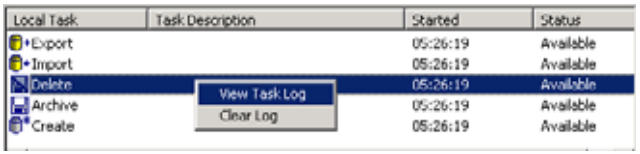
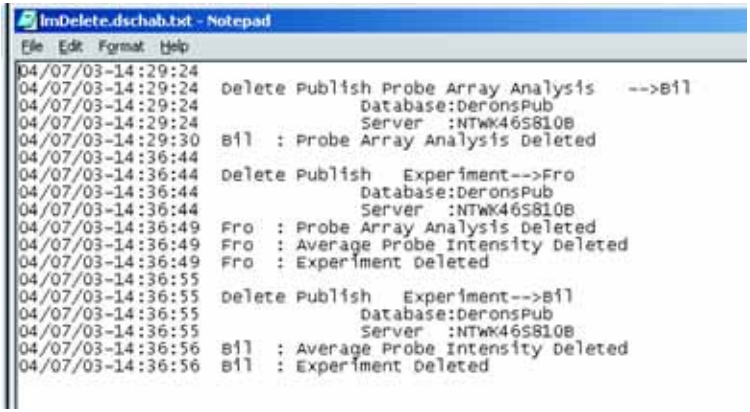


Figure 15.10
Task Status/Properties pane



```
ImDelete.dschab.txt - Notepad
File  Edit  Format  Help
04/07/03-14:29:24
04/07/03-14:29:24   Delete Publish Probe Array Analysis    -->Bil
04/07/03-14:29:24                Database:DeronsPub
04/07/03-14:29:24                Server   :NTWK46S810B
04/07/03-14:29:30   Bil  : Probe Array Analysis Deleted
04/07/03-14:36:44
04/07/03-14:36:44   Delete Publish    Experiment-->Fro
04/07/03-14:36:44                Database:DeronsPub
04/07/03-14:36:44                Server   :NTWK46S810B
04/07/03-14:36:49   Fro  : Probe Array Analysis Deleted
04/07/03-14:36:49   Fro  : Average Probe Intensity Deleted
04/07/03-14:36:49   Fro  : Experiment Deleted
04/07/03-14:36:55
04/07/03-14:36:55   Delete Publish    Experiment-->Bil
04/07/03-14:36:55                Database:DeronsPub
04/07/03-14:36:55                Server   :NTWK46S810B
04/07/03-14:36:56   Bil  : Average Probe Intensity Deleted
04/07/03-14:36:56   Bil  : Experiment Deleted
```

Figure 15.11
Delete task log

✔ Note

Deleting process data is different from deleting publish data (see ***Chapter 12*** Publishing Data on page 275*). Deleting published data removes only the data that has been published to a publish database. The data source in the process database remains available.*

Deleting a Probe Array Type

You can remove an unused or unwanted probe array type from the process database.

! CAUTION *Deleting a probe array type deletes the associated usersets. The data created with these usersets remain, but are not accessible.*

1. In the process pane data tree, expand the assay folder (for example, Expression) under the Probe Array Type folder **(Figure 15.12)**.

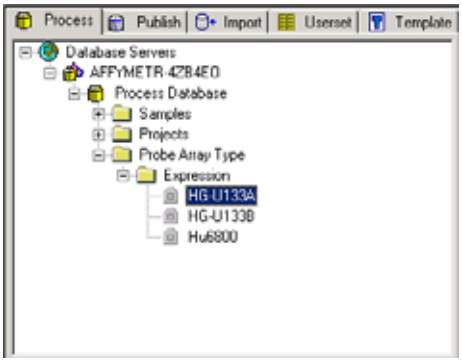


Figure 15.12
Process pane

2. Right-click the probe array type that you want to delete and select **Delete** from the shortcut menu that appears. Alternatively, click the probe array type and select **Process →Delete** from the menu bar.

   ⇒ A warning/confirmation box appears **(Figure 15.13)**.

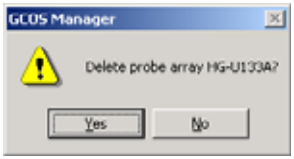


Figure 15.13
Delete warning/confirmation box

3. Click **Yes** to continue.

   ⇒ The selected probe array type is removed from the process database.

Exporting Data

You can export (copy) sample or experiment data (.dat, .cel, .chp) to an external storage location.

✔ Note *Sample and experiment information are exported to an Affymetrix® Microarray Suite 5.x .exp file.*

1. In the Data pane, right-click the item that you want to export and select **Export** from the shortcut menu that appears. Alternatively, select the item and click the **Data Path** button or select **Tools →Data Path** from the menu bar.

   Selecting an object includes all of the data that are associated with it. For example, selecting image data (.dat) includes any related cell intensity data (.cel) and probe analysis data (.chp).

   ⇒ The Select Export Data Path dialog box appears **(Figure 15.14)**.

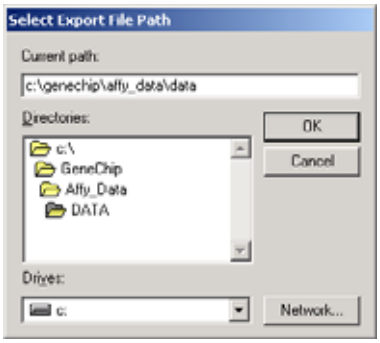


Figure 15.14
Select Export Data Path dialog box

2. Choose a drive from the Drive drop-down list and a directory path from the Directories box.

3. Click **Network** to map a network drive to a drive letter.

4. Click **OK**.

   ⇒ The Task Status/Properties pane displays the export local task status.

Note

To view more information about the export task status, right-click Export *in the Task Status/Properties pane and select* ***View Task Log*** *from the shortcut menu that appears* **(Figure 15.15)**. *Alternatively, double-click* Export *to view the export task log* **(Figure 15.16)**.

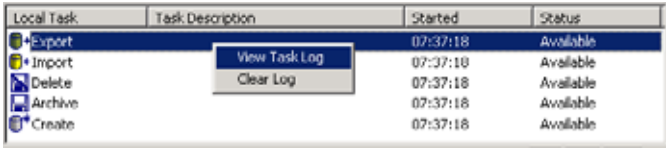


Figure 15.15
Task Status/Properties pane

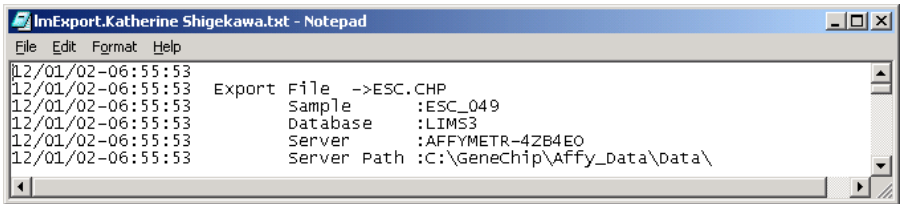


Figure 15.16
Export task log

Registering a GCOS Server

Registering a GCOS server makes it available to GCOS Manager.

1. Click the button or select **Tools →Register GCOS Server** from the menu bar.

   ⇒ The Register GCOS Server dialog box appears **(Figure 15.17)**.

Figure 15.17
Register GCOS Server dialog box

2. Select the server that you want to register and click **OK**.

   ⇒ The selected server is added to the drop-down list on the toolbar and becomes the active server.

   If the selected server is not GCOS aware, an error message is displayed **(Figure 15.18)**.

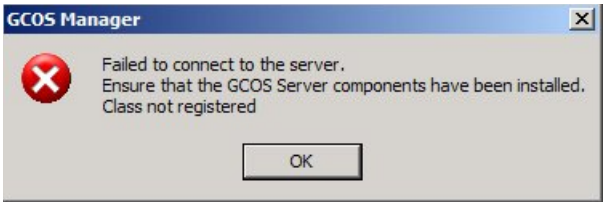


Figure 15.18
Register GCOS Server error message

Note

The error message indicates that there is no GCOS Server software installed, or that there is an installation error. For further information, please refer to the Affymetrix® GeneChip® Operating Software Server Installation and Administration Guide.

Unregistering a GCOS Server

- In the process pane, right-click the GCOS server that you want to remove and select **Unregister GCOS Server** from the shortcut menu that appears.

Viewing Server Events

The server events option displays a log of the events that occurred on the active server.

- To display the Event Viewer, click the **Server Events** button or select **Tools** →**Server Events** from the menu bar **(Figure 15.19)**.

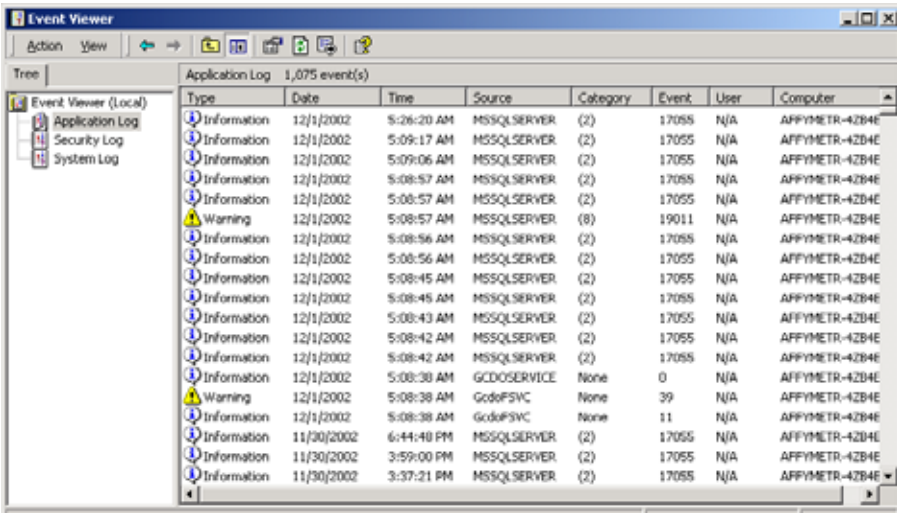


Figure 15.19
Event Viewer shows an event log for the active server

Viewing the Publish Tasks

You can use the Server Task option to view the publishing queue on the active server.

1. Click the **Server Tasks** button or select **Tools** →**Server Tasks** from the menu bar.

   ⇒ The task list for the active server appears and shows all items in the publishing queue **(Figure 15.20)**.

2. To sort the task list in ascending order, click a column header to define the primary sort order (for example, the Started column header). To sort the task list in descending order, click the same column header again.

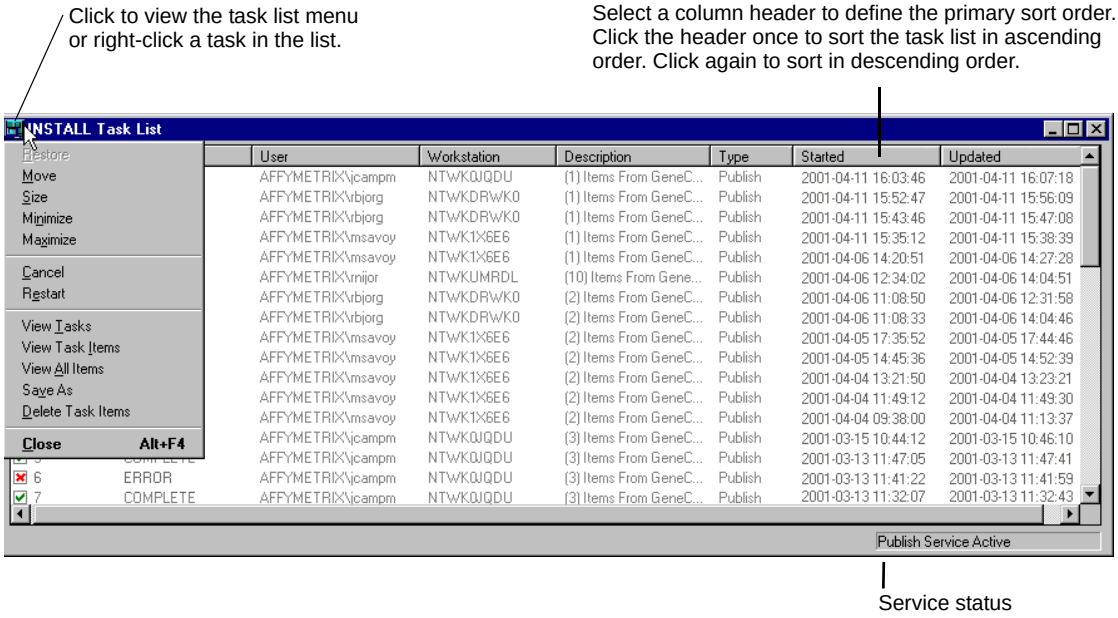


Figure 15.20
Task list displays the publishing queue on the active server

Task List Items & Commands

☐ indicates a new task in the queue waiting to be published

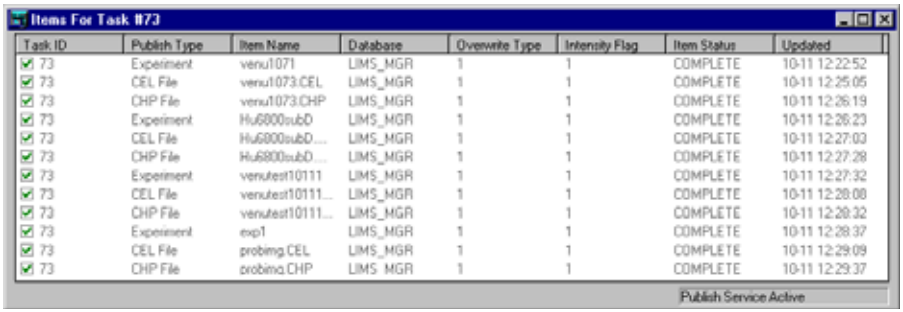
☑ blue check mark indicates a task that is being published

✅ green check mark indicates a completed (published) task

❌ indicates an incomplete task that was not published due to an error

To view the task list commands, click the icon (upper left corner of the window) **(Figure 15.20)** or right-click a task to display a shortcut menu.

Task List Menu Command	Use this command to...
View Tasks	Display the status of each task in the publishing queue on the active server (Figure **(Figure 15.21)**).
View Task Items	Display all items for a selected task **(Figure 15.21)**.
View All Items	Display all items for all tasks in the Task List window.
Cancel	Cancel a pending publish task.
Restart	Reactivate the publish process for a cancelled task.
Save As	Save the task list to a text file (.txt).
Delete Task Items	Remove all task items from the Task List window.


Items For Task #73

Task ID	Publish Type	Item Name	Database	Overwrite Type	Intensity Flag	Item Status	Updated
73	Experiment	venu1071	LIMS_MGR	1	1	COMPLETE	10-11 12:22:52
73	CEL File	venu1073.CEL	LIMS_MGR	1	1	COMPLETE	10-11 12:25:05
73	CHP File	venu1073.CHP	LIMS_MGR	1	1	COMPLETE	10-11 12:26:19
73	Experiment	Hu6800subD	LIMS_MGR	1	1	COMPLETE	10-11 12:26:23
73	CEL File	Hu6800subD....	LIMS_MGR	1	1	COMPLETE	10-11 12:27:03
73	CHP File	Hu6800subD....	LIMS_MGR	1	1	COMPLETE	10-11 12:27:28
73	Experiment	venutest10111	LIMS_MGR	1	1	COMPLETE	10-11 12:27:32
73	CEL File	venutest10111...	LIMS_MGR	1	1	COMPLETE	10-11 12:28:08
73	CHP File	venutest10111...	LIMS_MGR	1	1	COMPLETE	10-11 12:28:32
73	Experiment	exp1	LIMS_MGR	1	1	COMPLETE	10-11 12:28:37
73	CEL File	probimg.CEL	LIMS_MGR	1	1	COMPLETE	10-11 12:29:09
73	CHP File	probimg.CHP	LIMS_MGR	1	1	COMPLETE	10-11 12:29:37

Publish Service Active

Figure 15.21
Server Task window, View Task Items command displays all items for a selected task

Saving the Task List

You can save the items in the Server Task window to a text file (.txt).

1. Right-click a task in the Server Task window and select **Save As** from the shortcut menu that appears. Alternatively, click the icon in the upper left corner of the Task List window and select **Save As** from the drop-down menu.

   ⇒ The Save As dialog box appears **(Figure 15.22)**.

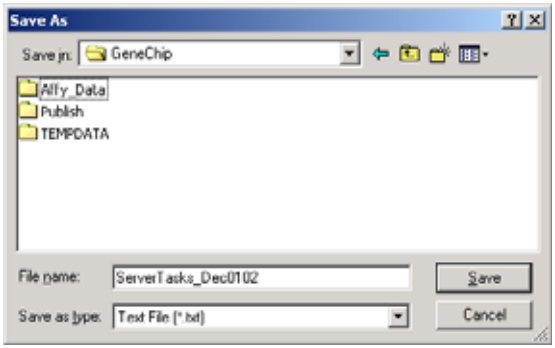


Figure 15.22
Save As dialog box

2. From the **Save in** drop-down list, navigate to the destination where you want to save the .txt file.

3. Name the file and click **Save**.

   ⇒ The task list is saved as a text file in the defined destination.

Deleting Task Items

To remove all of the task items from the Server Task Window:

1. Right-click a task and select **Delete Task Items** from the shortcut menu that appears. Alternatively, click the icon in the upper left corner of the Task List window and select **Delete Task Items** from the drop-down menu.

   ⇒ A warning/confirmation window appears **(Figure 15.23)**.

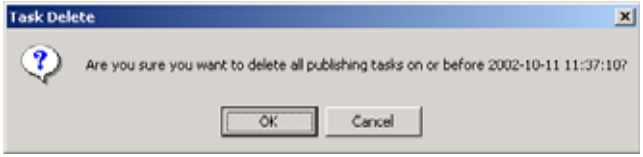


Figure 15.23
Task Delete warning/confirmation

2. Click **Yes** to delete all tasks on and before the date and time indicated.

   ⇒ The items are deleted and Server Task window is empty.

Cancelling or Restarting an Active Publish Task

- To stop the publish process, right-click a pending task and select **Cancel** from the shortcut menu that appears.
- To reactive the publish process for a task that has been cancelled or stopped due to error, right-click the task and select **Restart** from the shortcut menu that appears.

Find Function

You can use the **Find** feature to search the process database for samples, experiments, or experiment data (.dat, .cel, .chp), then apply the **Export**, **Archive**, **Assume Ownership** or **Delete** command to the objects returned by the search. The action affects only the selected objects and does not affect related children objects.

Note

*The **Find** function searches only the process database.*

1. Click the **Find** button. Alternatively, select **Tools** →**Find** from the menu bar.

   ⇒ The Find window appears **(Figure 15.24)**.

   The Find window has three tabs:

   - Select Object
   - Advanced Filter
   - Date Filter

Note

All three Find tabs work in union. For example, if you select sample (object type) in the Select Object tab, then only the boxes that pertain to finding samples will be active in the Advanced Filter tab.

You may open multiple Find windows.

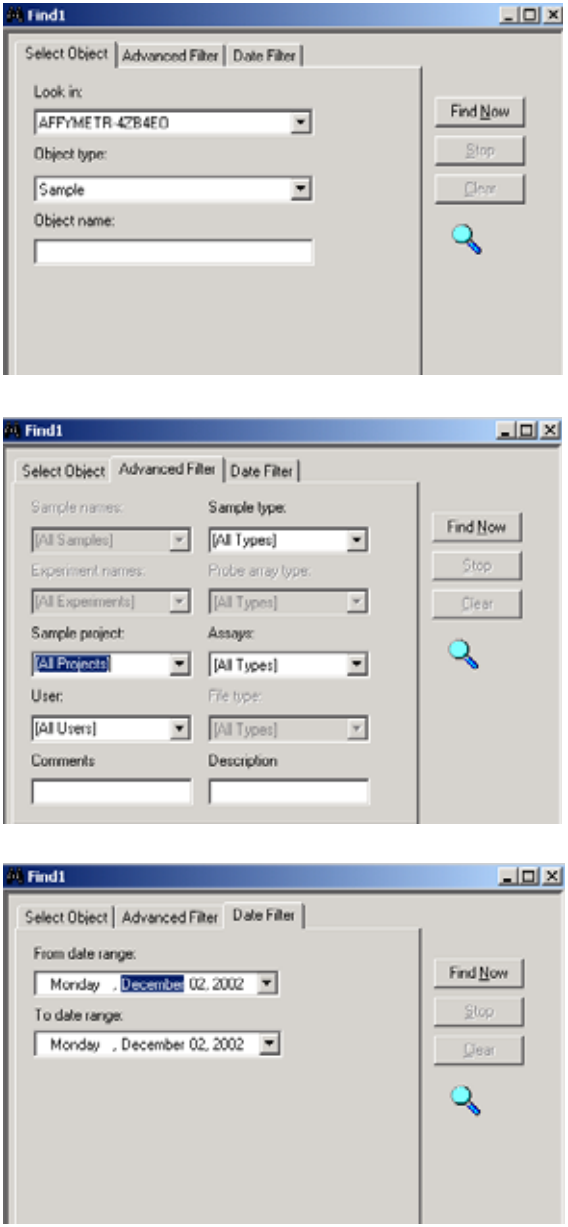


Figure 15.24
Find window, Select Object tab

2. Click the **Select Object**, **Advanced Filter**, or **Data Filter** tab and make selections from the drop-down lists, or enter text for the search.

   Further refine your search by specifying additional search parameters in the other Find tabs.

3. Click **Find Now**.

   ⇒ The search results are displayed at the bottom of the window **(Figure 15.25)**.

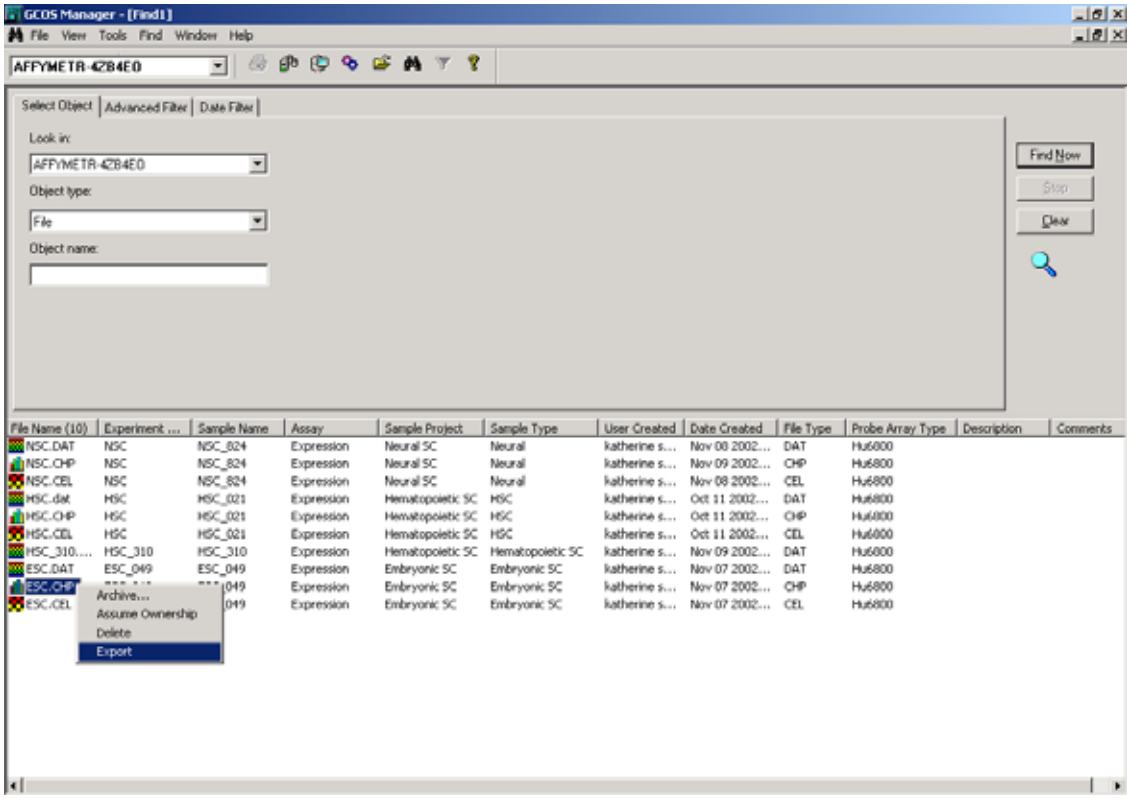


Figure 15.25
Find window, Select Object tab

4. To sort the search results, choose a column header to define the primary sort order. Click the column header to sort the results in ascending order. Click the header again to sort in descending order.

5. To apply the **Export**, **Archive/Unarchive**, **Delete**, or **Assume Ownership** command to the returned search objects:

   a. Select the objects for the command.

   To select adjacent objects, press and hold the **Shift** key, and click the first and last object in the selection. To select non-adjacent objects, press and hold the **Ctrl** key while you click the objects of interest.

   b. Right-click the selection and choose the command that you want to apply in the shortcut menu that appears **(Figure 15.25)**. Alternatively, select the command from the **Find** menu in the menu bar.

✔ Note

When you apply a command to selected search results, the command affects only the selected objects and does not affect any associated data (related children objects).

Filtering Samples

You can filter the samples that are displayed in the Process tab data tree **(Figure 15.26)**. Sample filters are available in the Process or Import tab when you click a subfolder of the Samples directory. The filters remain active from session to session until the parameters are changed.

1. In the data tree, click the Samples folder and the assay folder (for example, Expression).

   ⇒ The data tree displays the samples and the **Filter** button becomes available.

2. Click the **Filter** button.

   ⇒ The Sample Filter dialog box appears **(Figure 15.26)**.

3. Select filter parameters from the drop-down boxes.

   The **Role** and **User** filter options are mutually exclusive. You may select one or the other, but not both.

4. Click **Apply**.

   ⇒ The sample filters are applied to the data tree.

Specify sample filters that determine the samples displayed in the data tree.

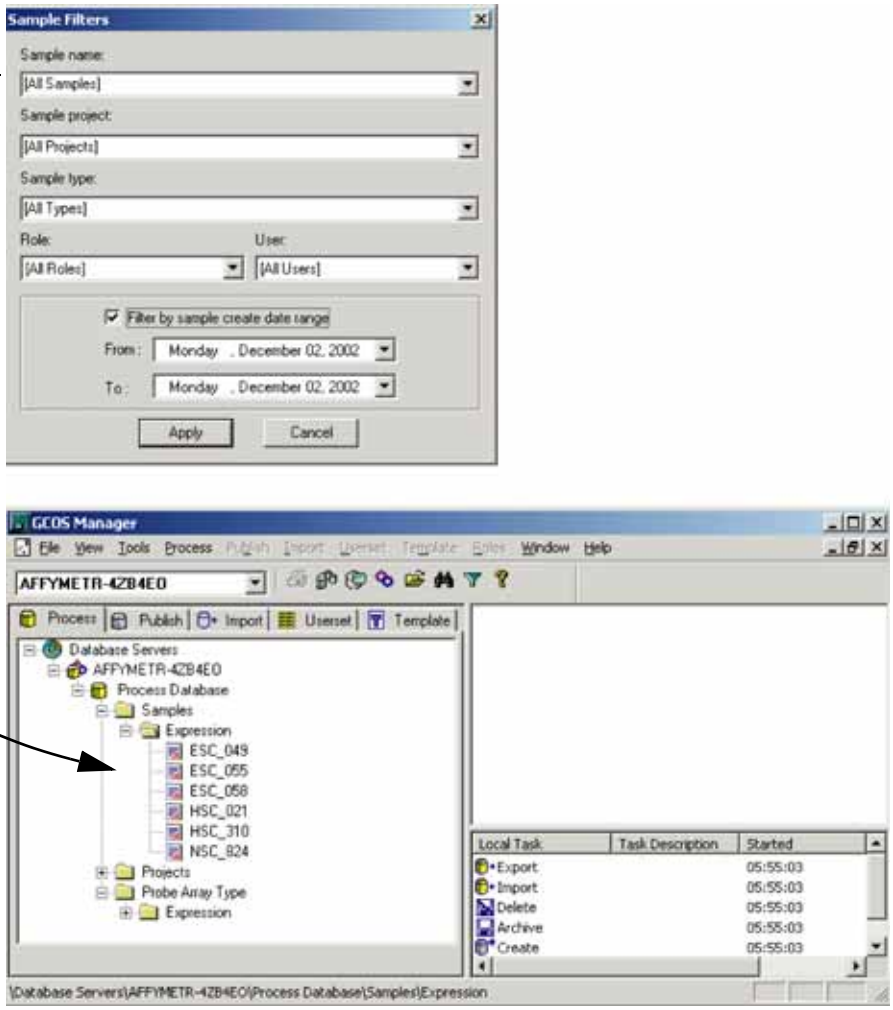


Figure 15.26
Selections in the Sample Filters dialog box (top) determine the samples displayed in the process pane data tree (bottom)

Publish Tab

In the Publish tab you can create new publish databases and delete published data from a database. This section of the manual explains the layout of the Publish tab window, its functions, and how to accomplish tasks here.

- Click the Process tab.

⇒ The three Process tab windowpanes are displayed **(Figure 15.27)**.

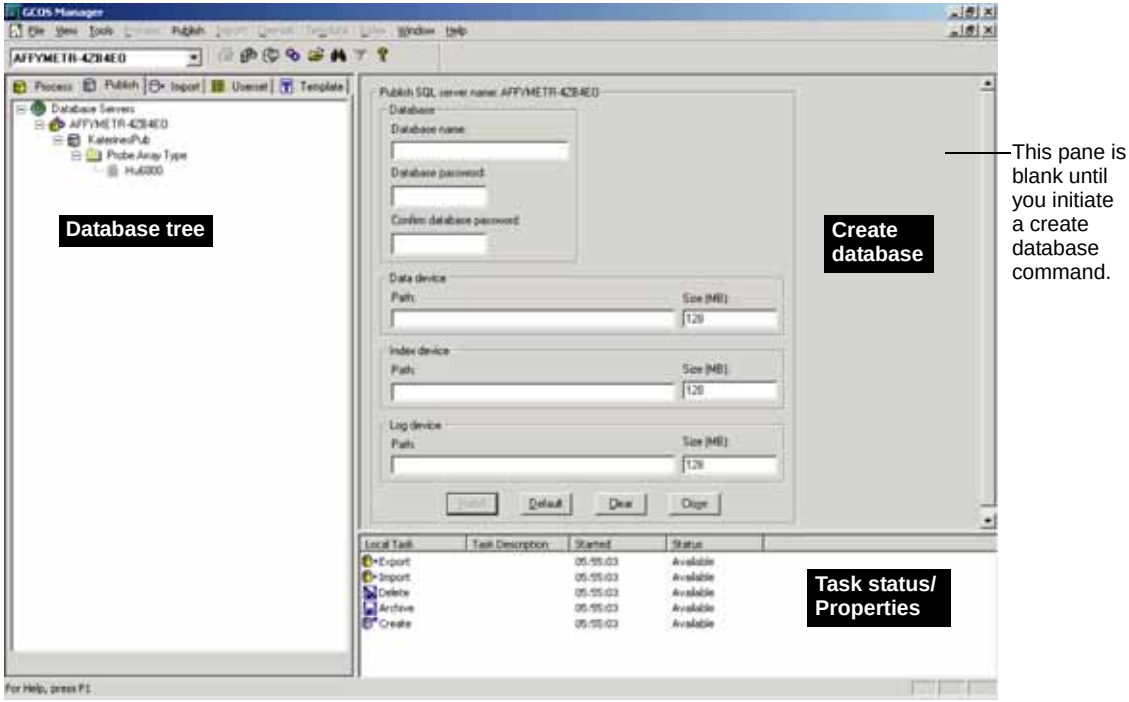


Figure 15.27
Publish tab windowpanes

Publish Tab Windowpanes

Publish database tree	Displays a tree that shows the active server, the publish databases on the server, and the supported probe array types and their contents experiment data and analysis data (.cel, .chp) that have been published for that probe array type).
Create database	Shows the Create Database dialog box when you initiate a create database command.
Task Status/ Properties pane	Displays the status of processes that may be occurring in the database (for example, export, import, delete, archive, or create). In the task status view, you can monitor these jobs while you work on other tasks in the system.

Publish Tab Functions

The primary tasks in the Publish tab are:

- create a publish database
- delete data or a probe array type from the publish database

The tasks associated with a publish database, published data, or a probe array type are found under **Publish** in the menu bar. Alternatively, right-click an item to view a shortcut menu that appears **(Figure 15.28)**.

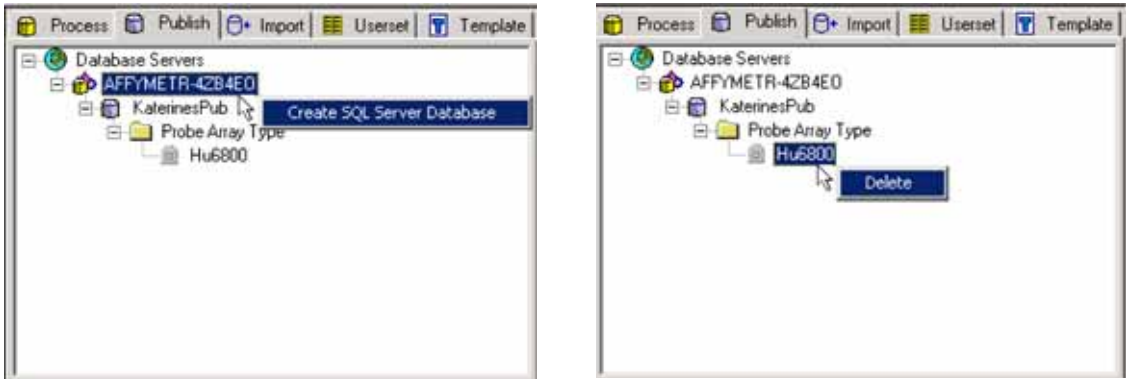


Figure 15.28
Publish tab shortcut menus

Accessing a Publish Database

Each publish database is password protected. The password is defined during the creation of the database. You can change the password for a database on the GCOS server using the GCOS Server Sync utility. (For more information on the GCOS Server Sync utility, see the *Affymetrix® GeneChip® Operating Software Server Installation and Administration Guide*.)

1. In the publish pane, click the database that you want to access.

   ⇒ A Publish Database Login window appears **(Figure 15.29)**.

2. Enter the database password and click **Login**.

✔ Note *Passwords are case-sensitive.*

   ⇒ The publish database tree expands and displays the Probe Array Type folder **(Figure 15.29)**.

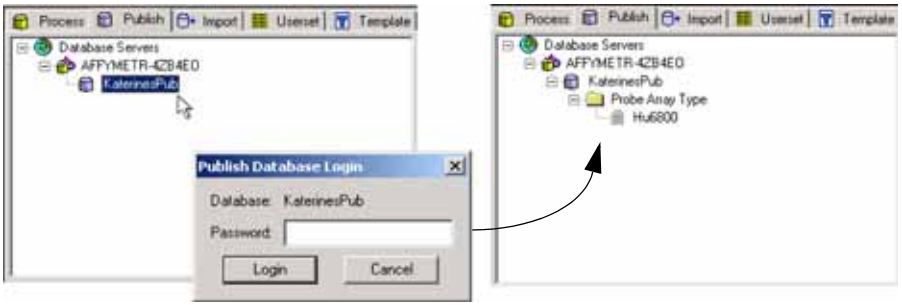


Figure 15.29
Publish Database Login box

Deleting a Probe Array Type

Probe array types that are no longer used may be removed from a publish database. When you delete a probe array type, all of the associated published data (from the parent down) are also deleted.

1. Click the Probe Array Type folder under the publish database of interest.
2. Right-click the probe array type that you want to delete and select **Delete** from the shortcut menu that appears. Alternatively, click the probe array type and select **Publish →Delete** from the menu bar.

   ⇒ A warning window appears **(Figure 15.30)**.

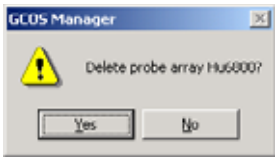


Figure 15.30
Probe array delete warning/confirmation box

3. Click **Yes** to continue.

   ⇒ The probe array type and all associated data are removed from the publish database.

Deleting Published Data

Deleting publish data removes selected cell intensity or probe analysis data from the publish database. When you delete published data, all children data are also removed.

! CAUTION

Deleting publish data permanently removes the data from the publish database. If the information still resides on the process database, the data can be restored to the publish database by repeating the publish procedure in GCOS.

1. In the server drop-down list, select the server where the publish database resides.
2. In the publish pane, select the database of interest and expand the tree.
3. Right-click the data that you want to delete and select **Delete** from the shortcut menu that appears. Alternatively, click the data and select **Publish →Delete** from the menu bar.

4. On the menu bar select **Publish →Delete**.

   ⇒ A warning/confirmation box appears **(Figure 15.31)**.

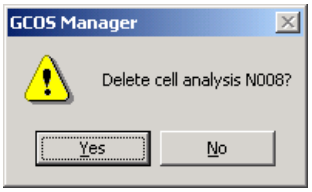


Figure 15.31
Analysis delete warning / confirmation window

5. Click **Yes** to continue.

   ⇒ The **Delete** task proceeds.

   The task status/properties pane displays the status of the delete task.

✔ Note

To view more information about the delete task status, right-click Delete *in the Task Status/Properties pane and select* ***View Task Log*** *from the shortcut menu that appears* **(Figure 15.32)**. *Alternatively, double-click* Delete *to view the delete task log* **(Figure 15.33)**.

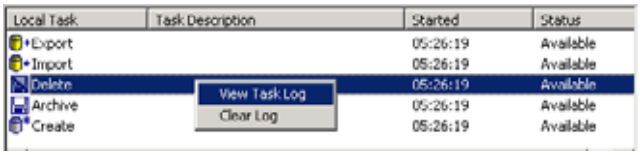
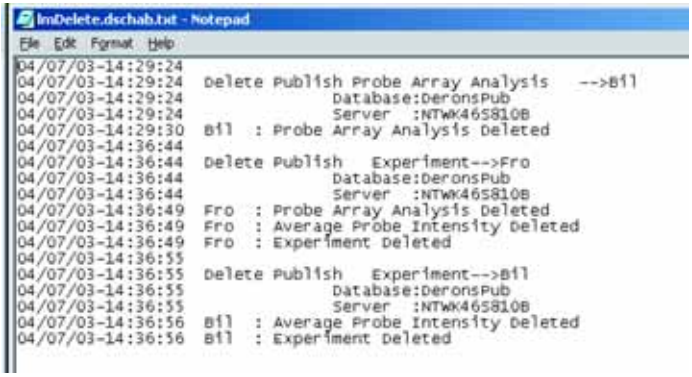


Figure 15.32
Task Status/Properties pane


InDelete.dschab.txt - Notepad

File Edit Format Help

```
04/07/03-14:29:24
04/07/03-14:29:24  Delete Publish Probe Array Analysis   -->Bil
04/07/03-14:29:24                Database:DeronsPub
04/07/03-14:29:24                Server  :NTWK46S810B
04/07/03-14:29:30  Bil  : Probe Array Analysis Deleted
04/07/03-14:36:44
04/07/03-14:36:44  Delete Publish   Experiment-->Fro
04/07/03-14:36:44                Database:DeronsPub
04/07/03-14:36:44                Server  :NTWK46S810B
04/07/03-14:36:49  Fro  : Probe Array Analysis Deleted
04/07/03-14:36:49  Fro  : Average Probe Intensity Deleted
04/07/03-14:36:49  Fro  : Experiment Deleted
04/07/03-14:36:55
04/07/03-14:36:55  Delete Publish   Experiment-->Bil
04/07/03-14:36:55                Database:DeronsPub
04/07/03-14:36:55                Server  :NTWK46S810B
04/07/03-14:36:56  Bil  : Average Probe Intensity Deleted
04/07/03-14:36:56  Bil  : Experiment Deleted
```

Figure 15.33
Delete task log

Creating a Publish Database

In the Publish tab, you can create a new publish database. **Table 15.10** shows the location, size, and storage capacity options for a publish database.

Along with the new database, GCOS Manager also creates the ODBC DSNs that are required for publishing data. Several key pieces of information are needed to create a publish database, including:

- the server where the database will reside
- a username and password with appropriate permissions for creating databases on the selected server
- the database size
- the proper drive and path for the database files

The server name is represented by an ODBC data source name description. This ODBC DSN specifies the location of an administration database that is used to create the actual publish database. This DSN is usually created during installation of the software.

The initial size of a Microsoft® SQL Server 2000 publish database on the GCOS server can range from 128 to 1024 MB. The size can easily be increased after the database is created. For information on increasing the size of a publish database on the GCOS server, see the *GCOS Server Administration and Installation Guide*.

A MSDE publish database on the workstation has a 2 GB size limit. If the database reaches this limit, create a new publish database.

Note *MSDE cannot create a publish database on a network drive.*

Table 15.10
Publish database location, size, and storage capacity options

Publish Database/ Location	Size Options	Storage Capacity[a]
Microsoft® MSDE/ workstation	up to 2 GB	8 MB per experiment (includes experiment information, .cel, .chp) 8 MB per library file 128 MB database = 4 library files & 12 experiments
Microsoft® SQL Server 2000/GCOS server	128-1024 MB initial size range	8 MB per experiment (includes experiment information, .cel, .chp) 8 MB per library file 128 MB database = 4 library files & 12 experiments 1024 MB database = 16 library files & 110 experiments
Oracle®/GCOS server	Small (~6 GB) Large	~500 experiments ~1000 experiments

a. Based on a probe array with 270x270 features, 1800 probe sets per array.

Creating a Microsoft® MSDE Publish Database on the Workstation

1. Select the server where the publish database will reside from the drop-down list on the toolbar **(Figure 15.34)**.
2. In the publish pane, right-click the server name in the data tree and select **Create MSDE Database** from the shortcut menu that appears. Alternatively, click the server name and select **Publish →Create MSDE Database** from the menu bar.

   ⇒ The Create Database dialog box appears **(Figure 15.34)**.

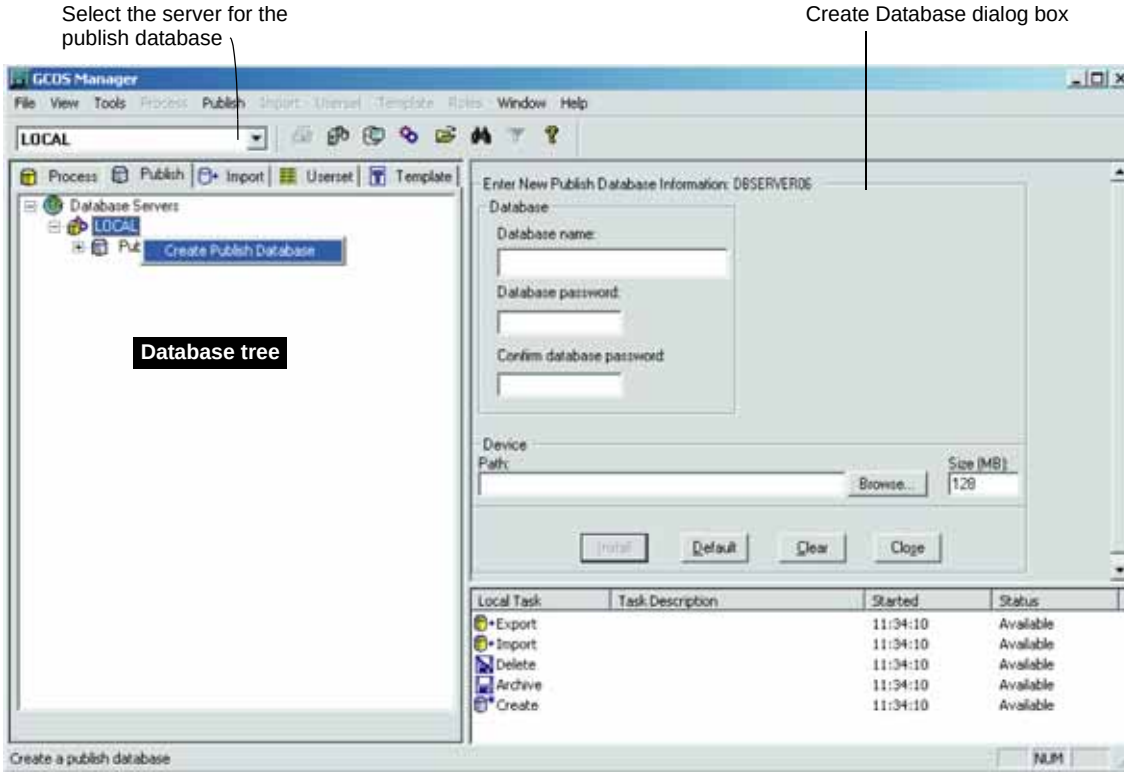


Figure 15.34
Publish tab, create a MSDE publish database

3. In the Create Database dialog box:

   a. Enter a name for the new database.

   The database name is limited to 22 characters in length.

   b. Enter a password for the database. Re-enter the password to confirm it.

   The publish database can only be accessed with this password.

4. To select the path for the database, do either of the following:

   - Enter the path for the publish database

- Click **Browse** and select a folder from the Choose Folder dialog box **(Figure 15.35)**.

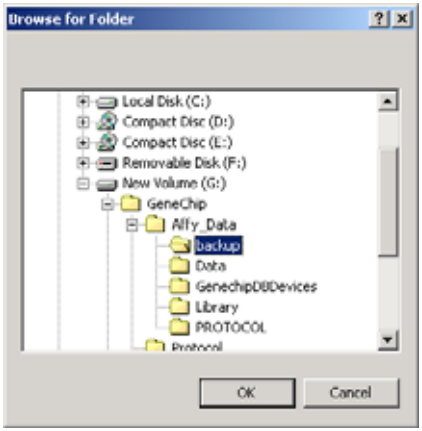


Figure 15.35
Choose Folder dialog box

5. Click **Install**.

   ⇒ It takes several minutes to create the database and allocate the amount of drive space requested (2 -15 minutes or more, depending on the speed of the workstation and drive).

   Once it is created, the database is available for publishing without further configuration.

Creating a Microsoft® SQL Server 2000 Publish Database on the GCOS Server

1. Select the server where the publish database will reside from the drop-down list on the toolbar **(Figure 15.36)**.

2. In the database tree, right-click the server name and select **Create SQL Server Database** from the shortcut menu that appears. Alternatively, click the server name and select **Publish →Create SQL Database** from the menu bar.

   ⇒ The Create Database dialog box appears **(Figure 15.36)**.

3. In the Create Database dialog box:

a. Enter a name for the new database.

The database name is limited to 22 characters in length.

b. Enter a password for the database. Re-enter the password to confirm it.

The publish database can only be accessed with this password.

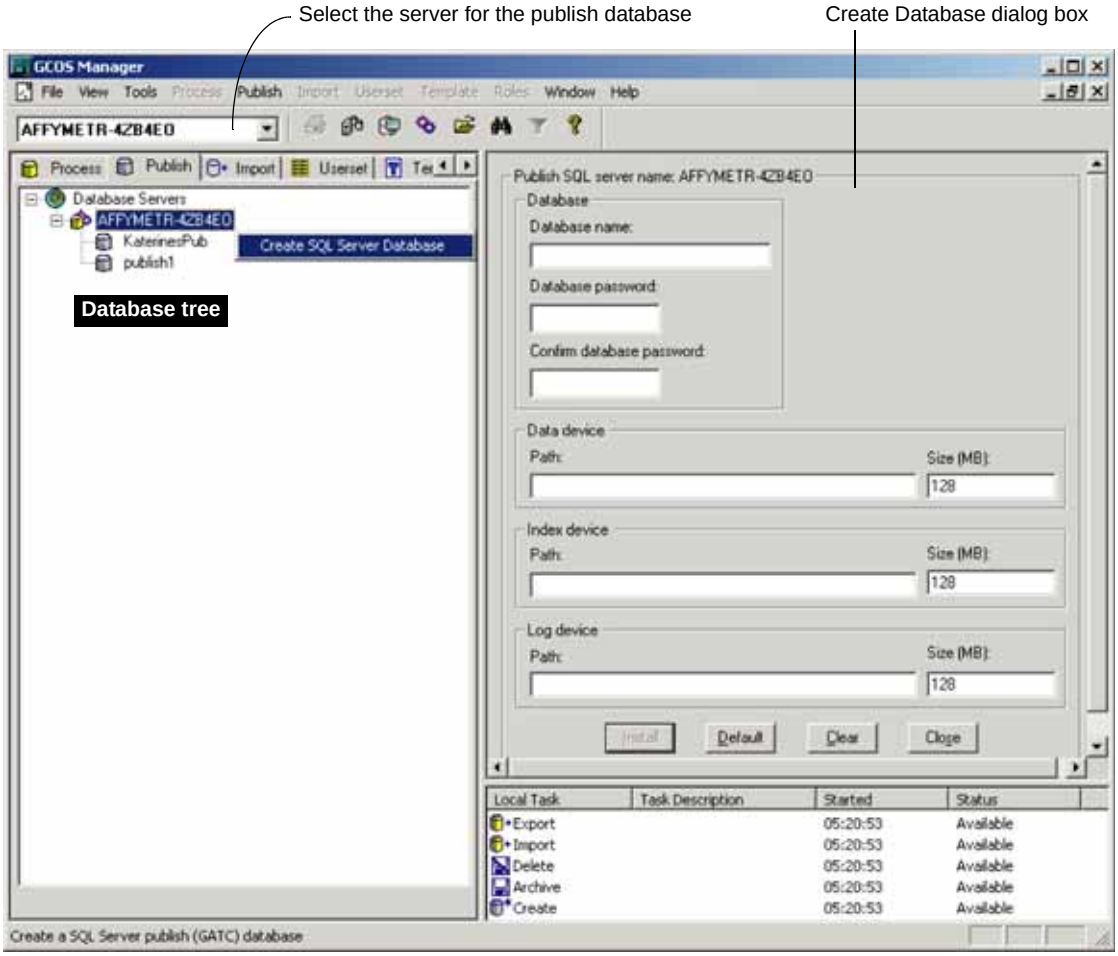


Figure 15.36
Publish tab, create a Microsoft® SQL Server publish database

4. Enter the following information in the **Data device**, **Index device**, and **Log device** sections:
   - **Path**: Use the complete drive description, including the drive letter, colon, and backslash (for example, E:\Affymetrix DBDevices).

✔ Note

The drive letter specifies the drive on the actual publish database server. If you are creating the publish database on a remote server, make sure the selected drive letter is available on that server.

   - **Size (MB)**: The size depends on the number of experiments that will be published to the database and the amount of available space on the selected drive. A Microsoft® SQL Server database is easy to expand after creation; therefore, creating a smaller database and expanding it as necessary is recommended.
5. Click **Install**.

   It takes several minutes to create the database and allocate the amount of drive space requested (12-15 minutes or more, depending on the speed of the server and drive).

   Once created, the database is available for publishing without further configuration. If the new database does not appear in the data tree, verify that the database and ODBC DSN were created properly.

Creating an Oracle® Publish Database on the GCOS Server

An Oracle publish database is much larger than the default Microsoft SQL Server database. Before you create an Oracle database, verify that there is adequate space available on the server. The small size Oracle publish database requires 6 GB and the large size requires 12 GB. These sizes have capacity for approximately 500 and 1,000 published experiments respectively.

1. Select the server where the publish database will reside from the drop-down list on the toolbar **(Figure 15.37)**.
2. In the database tree, right-click the server name in the data tree and select **Create Oracle Database** from the shortcut menu that appears. Alternatively, click the server name and select **Publish →Create Oracle Database** from the menu bar.

   ⇒ The Create Database dialog box appears **(Figure 15.37)**.
3. In the Create Database dialog box:

a. Enter a name for the new database.

The database name is limited to 22 characters in length.

b. Enter a password for the database. Re-enter the password to confirm it.

The publish database can only be accessed with this password.

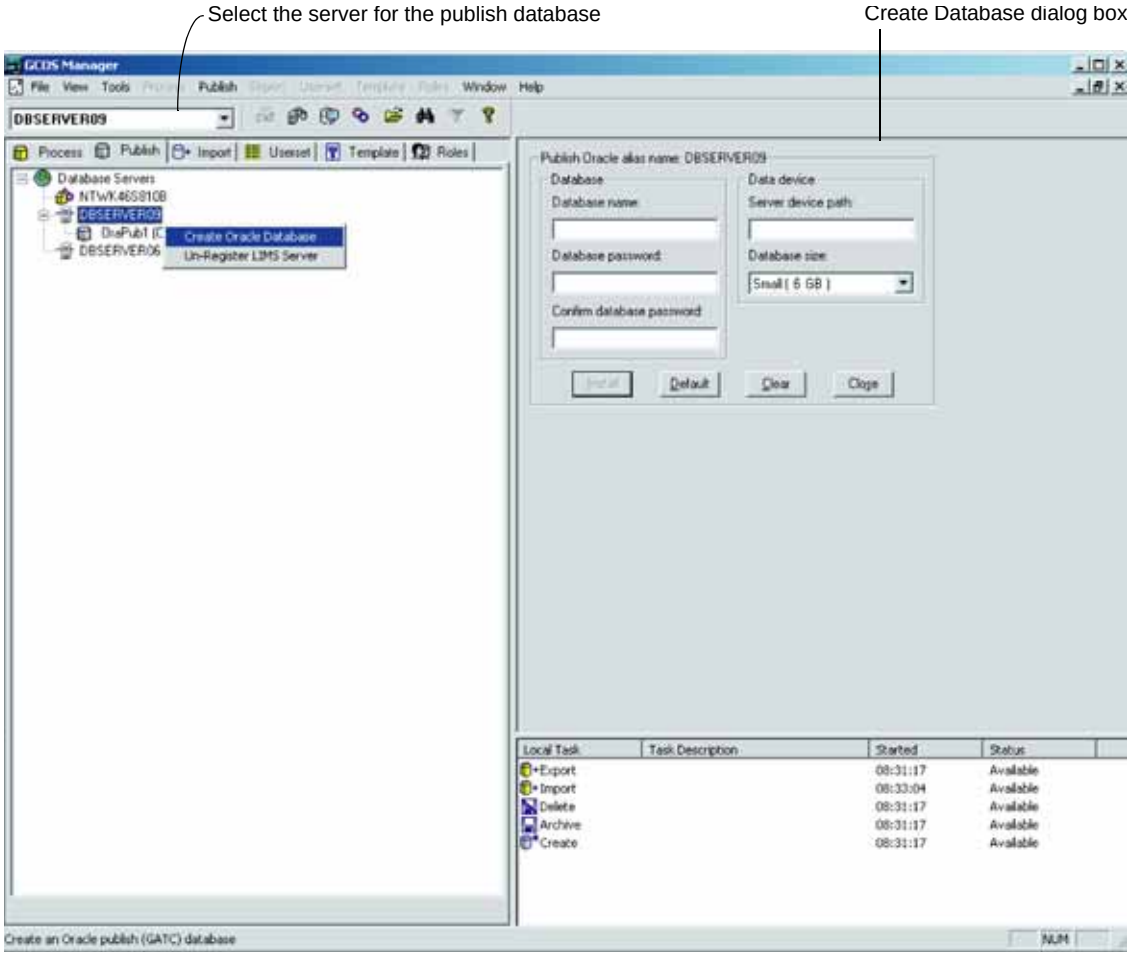


Figure 15.37
Publish tab, create Oracle® database

4. In the **Data Device** section enter the following information:
   - **Server Device Path**: This is the complete path for the database files. Use the complete drive description for Windows 2000 (for example, E:\Affymetrix DBdevices\). Be sure to include the trailing slash.

✔ Note

The data file path directly specifies the file location on the actual publish database server. If you are creating a publish database on a remote server, make sure the specified path is available on that server.

✔ Note

The data file path directory used specifies the file location on the actual publish database server. If the publish database is being created on a remote server, make sure the path specified is available on that server.

   - **Database Size**: make a selection from the drop-down list.
     The **Small** database requires approximately 6 GB of drive space and the **Large** requires approximately 12 GB.

5. Click **Install**.

   It takes several minutes to create the database and allocate the amount of drive space requested (15 - 30 minutes or more, depending on the speed of the server and drive).

   Once it is created, the database is available for publishing without further configuration. If the newly created database does not appear in the data tree, verify that the database and the ODBC DSN were created properly.

Import Tab

In the Import tab you can copy experiment data (.exp, .cel, .chp created in MAS 5.x) into the GCOS or GCOS server process database. This section of the manual explains the layout of the Import tab window and how to accomplish tasks here.

- Click the Import tab.

⇒ The four windowpanes of the Import tab are displayed **(Figure 15.38)**.

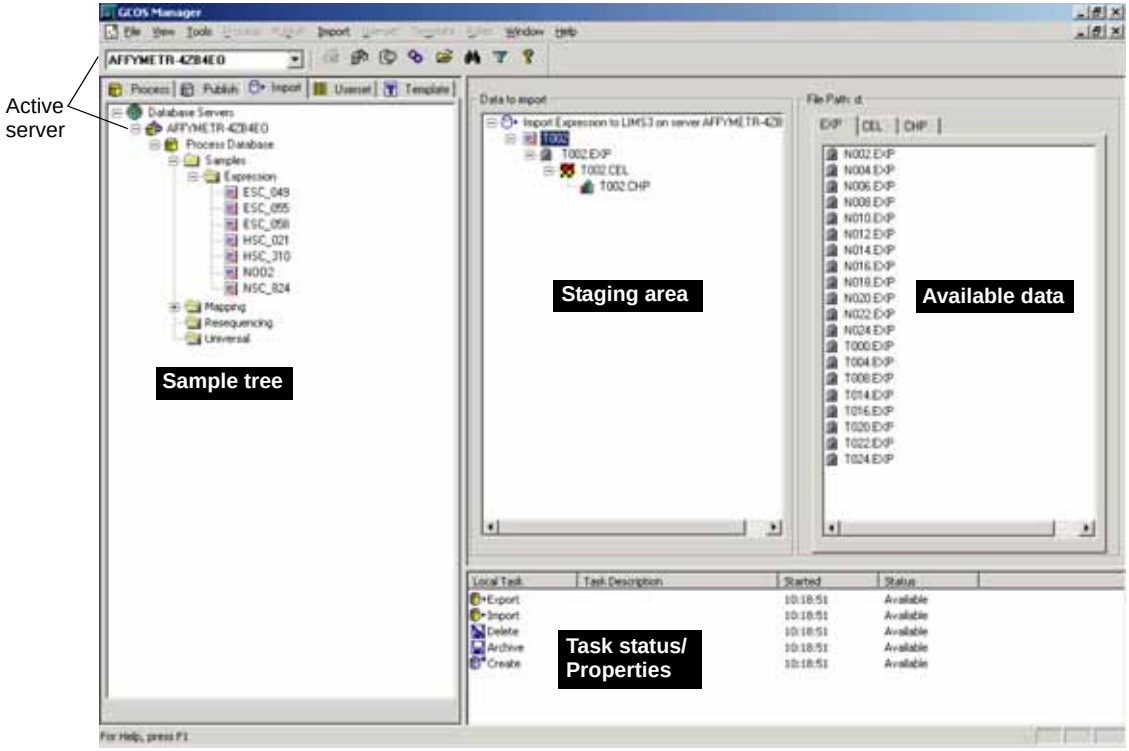


Figure 15.38
Import tab windowpanes

Filtering Samples

You can filter the samples that are displayed in the sample tree **(Figure 15.38)**. Sample filters are available in the Import or Process tab when you click a subfolder of the Samples directory. The filters remain active from session to session until the parameters are changed.

1. In the sample tree, click the Samples folder and the assay folder (for example, Expression).

   ⇒ The sample tree displays the samples and the **Filter** button becomes available.

2. Click the **Filter** button .

   ⇒ The Sample Filter dialog box appears **(Figure 15.26)**.

3. Select filter parameters from the drop-down boxes.

   The **Role** and **User** filter options are mutually exclusive. You may select one or the other, but not both.

4. Click **Apply**.

   ⇒ The sample filters are applied to the data tree.

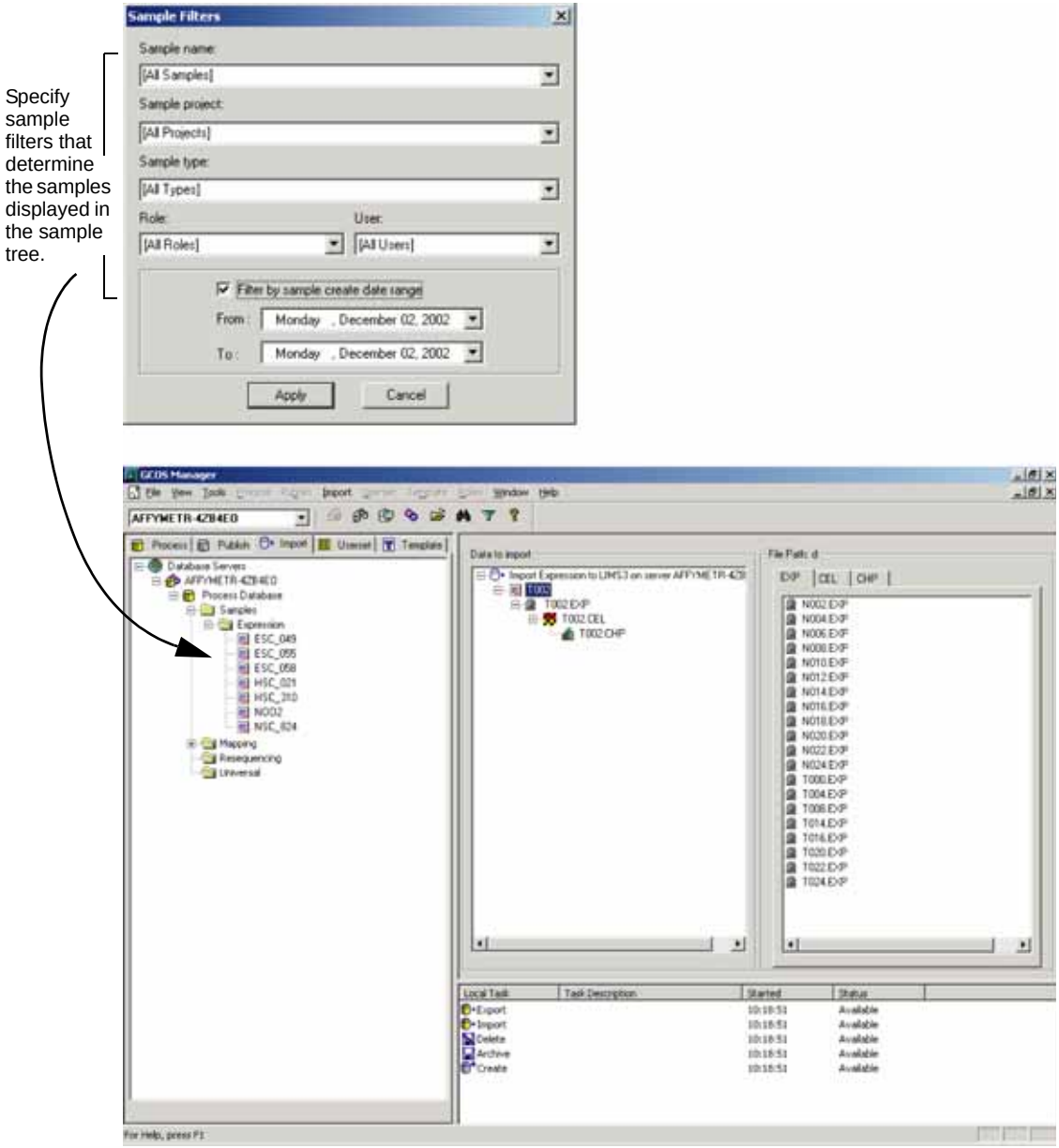


Figure 15.39
Selections in the Sample Filters dialog box (top) determine the samples displayed in the sample data tree (bottom)

Import Tab Windowpanes

Sample tree	Shows the active server, process database, and samples in the system.
Staging area	Shows the sample(s) that will be associated with experiment data for import.
Data available for import	Displays the experiment data located at the defined import data path.
Task status/ properties status/ Properties	Displays the property information or local task view, depending on the item selected in process database tree or the selected data pane. Property information is defined by the template that was used during sample registration in GCOS. The task status view shows the status of processes that may be occurring in the database (for example, export, import, delete, archive, or create). In the task status view, you can monitor these jobs while you work on other tasks in the system.

Importing Data

The Import function imports or copies experiment data created in MAS 5.x and moves them to a process database on the workstation or a GCOS server. You can import data to multiple GCOS servers.

Importing data is a three-step process:

- Select the data path that specifies the location of the MAS 5.x experiment data (.exp, .dat, .cel, .chp) that you want to import.
- Create a new sample or select an existing sample to associate with the experiment data (the data will be also be associated with the project specified by the sample information).
- Associate the experiment data with the selected sample so that GCOS Manager can track the data in the process database.

Selecting an Import File Path

1. Click the data path button . Alternatively, select **Tools →Data Path** from the menu bar.

   ⇒ The Select Import Data Path dialog box appears **(Figure 15.40)**.

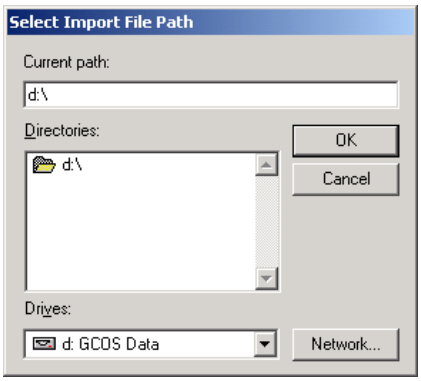


Figure 15.40
Select Import Data Path dialog box

2. Make a selection from the **Drives** from the drop-down list and navigate to the data that you want to import.

3. Click **OK**.

   ⇒ The defined data path and the experiment data at that location are displayed in the available data windowpane (upper right) in the Import tab **(Figure 15.41)**.

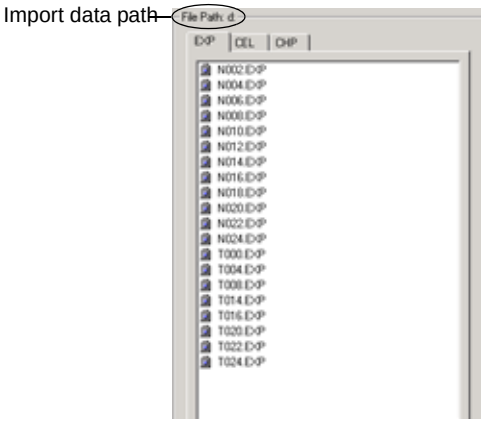


Figure 15.41
Import tab, available data windowpane shows data available for import at the current import data path

Note *The default data path is the same path assigned to Affymetrix® GCOS experiment data.*

Specifying a Sample for Imported Data

The experiment data for import are not associated with a sample or project. You must associate the data with a sample and a project so that GCOS Manager can track the data in the process database. You can associate the imported data with a new sample or an existing sample.

Creating & Editing a New Sample

1. Right-click the appropriate assay folder (for example, Expression) in the process database tree and select **Create Sample** from the shortcut menu that appears. Alternatively, click the assay folder and select **Import** → **Create Sample** from the menu bar.

   ⇒ The Create Sample dialog box appears **(Figure 15.42)**.

Table 15.11 and **Figure 15.14** list the data entry rules for the fields in the Create Expression Sample dialog box.

Table 15.11
Rules for data input

Data Input	Rule
Character length	≤ 64 characters
Invalid characters	\ / : * ? “ < > | ‘ ~ ´, { } [ ]
Required data fields	Required fields are indicated by blue titles and may not be left blank.
Data fields	A name may not duplicate an existing name.

Table 15.12
Valid field lengths

Sample Attribute	Length (characters)
Sample project	64
Sample type	64
Description	64
Comments	64
Sample name	64

2. Enter the following required information (or make selections from the drop-down lists):
   - Sample name
   - Sample project name
   - Sample type
3. Enter any descriptions or comments.

✔ Note

If the sample name, project name, and sample type do not match the information stored in the experiment file, the software notifies you and directs you to the import log to determine inconsistencies.

4. Click **OK**.
   ⇒ The new sample appears in the import staging area (middle pane).

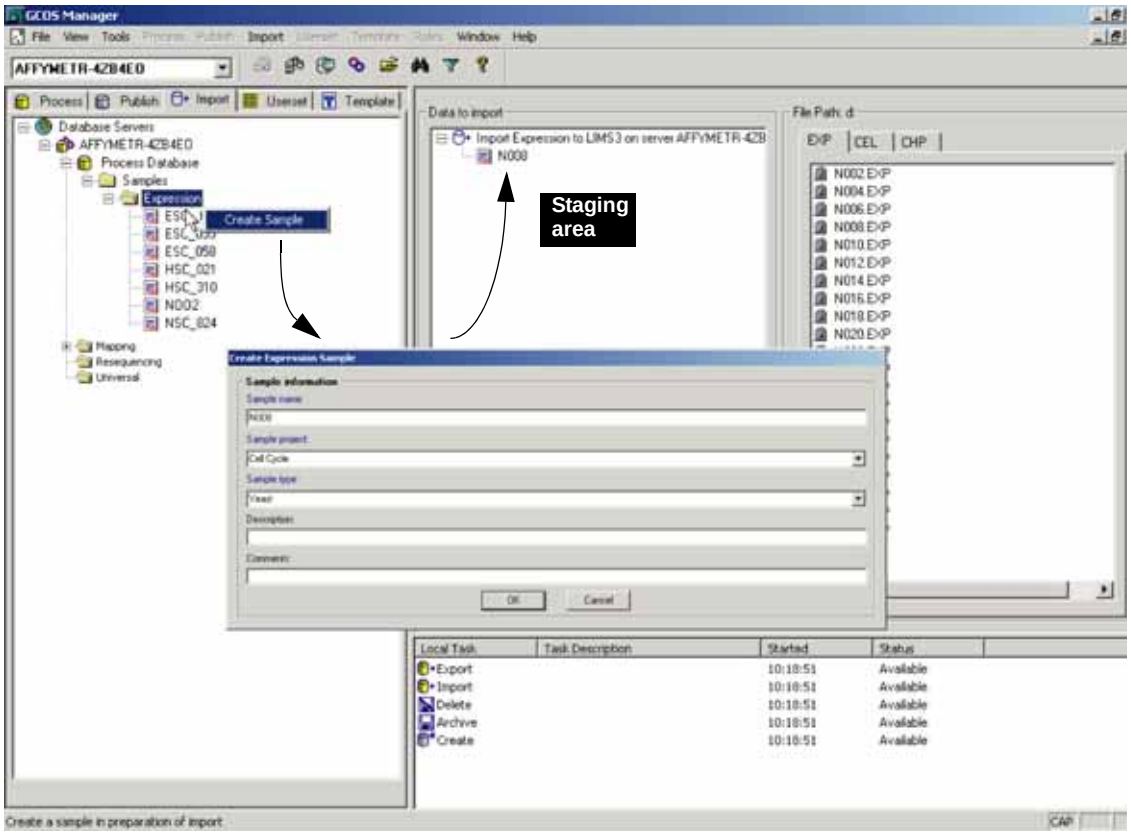


Figure 15.42
Import tab window and Create Sample dialog box

If necessary, you can edit the information for a new sample before you start the data import.

Note

The sample name cannot be edited. You can only edit a new sample prior to data import. After data import, a sample cannot be edited. Samples created in GCOS cannot be edited.

5. To edit the sample information prior to data import:

   a. Right-click the sample name and select **Edit** from the shortcut menu that appears **(Figure 15.43)**. Alternatively, click the sample name and select **Import →Edit** from the menu bar.

      ⇒ The Create Sample dialog box appears **(Figure 15.42)**.

Figure 15.43
Sample for imported data, shortcut menu

   b. Enter the new information in the Create Sample dialog box and click **OK** when finished.

Selecting an Existing Sample

1. In the process database tree, expand the sample folder and click the sample of interest **(Figure 15.44)**.

2. Drag the sample to the staging area.

   ⇒ The sample and all of the associated data are displayed with red check marks that indicate the data already exist in the database.

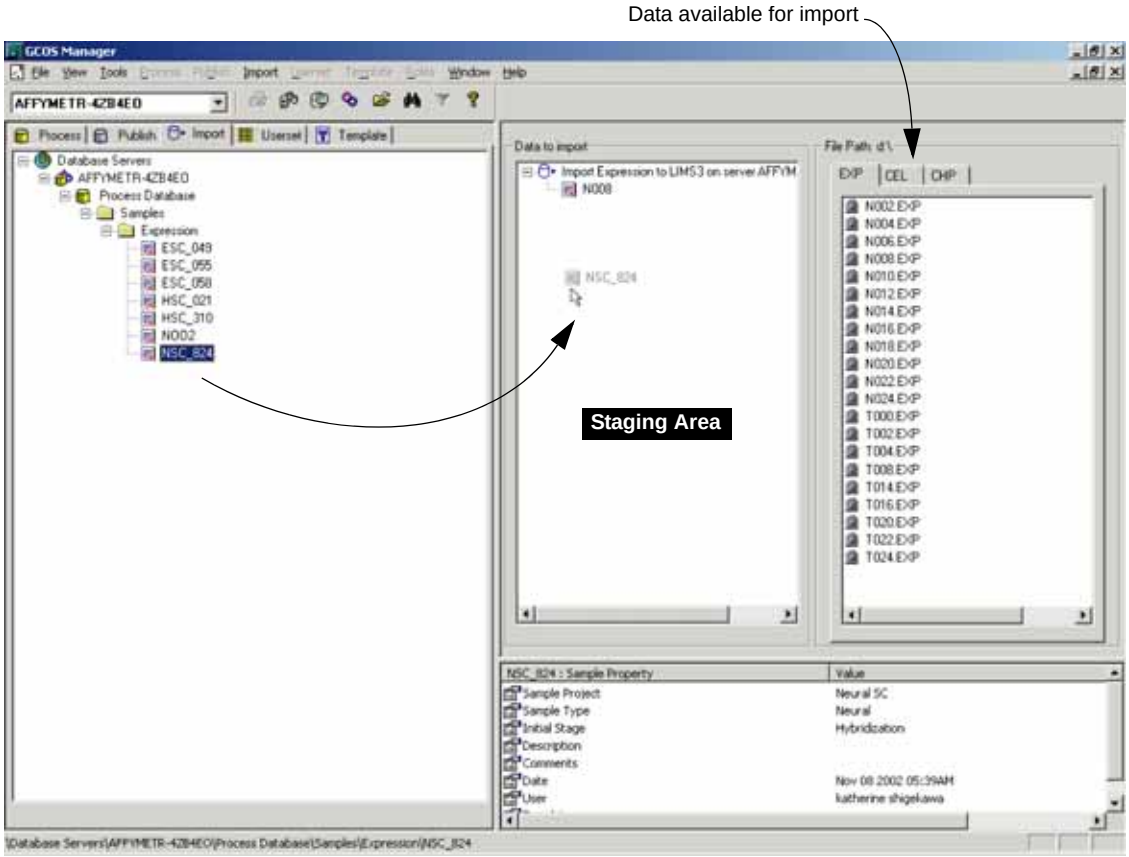


Figure 15.44
Import tab, drag an existing sample to the Staging Area

Removing a Sample From the Staging Area

You can remove samples from the Staging Area.

- Right-click the sample that you want to remove, and select **Remove** (for new samples) or **Remove All** (for existing samples) from the shortcut menu that appears **(Figure 15.43)**. Alternatively, click the

sample and select **Import →Remove** or **Import →Remove All** from the menu bar.

⇒ A new sample or an existing sample and its children files are removed from the staging area.

Associating Data & Samples

The last step in the import process is to associate the experiment data (.cel, .chp) with a sample. You can only associate data from probe array types installed in the process database. If you attempt to associate data from a probe array type that is not installed in the process database, an error is displayed.

✔ Note

The names of imported data must conform to the naming conventions in GCOS (see **Table 15.12** on page 325*). If necessary, rename a file so that the name complies with the naming conventions.*

Associating .exp with a Sample

In the available data pane **(Figure 15.45)**, the EXP tab displays the experiment data (.exp) available for import at the designated data import path. When you associate an experiment (.exp) with a sample, the .dat, .cel, and .chp with the same prefix (if they exist) are also automatically associated with the sample.

1. To associate a .exp with a sample, drag the data to the sample in the staging area **(Figure 15.45)**.

   ⇒ The .exp is added to the below the sample along with the .cel and .chp with the same prefix **(Figure 15.46)**.

2. To associate another .exp and its children data with the sample, repeat step 1.

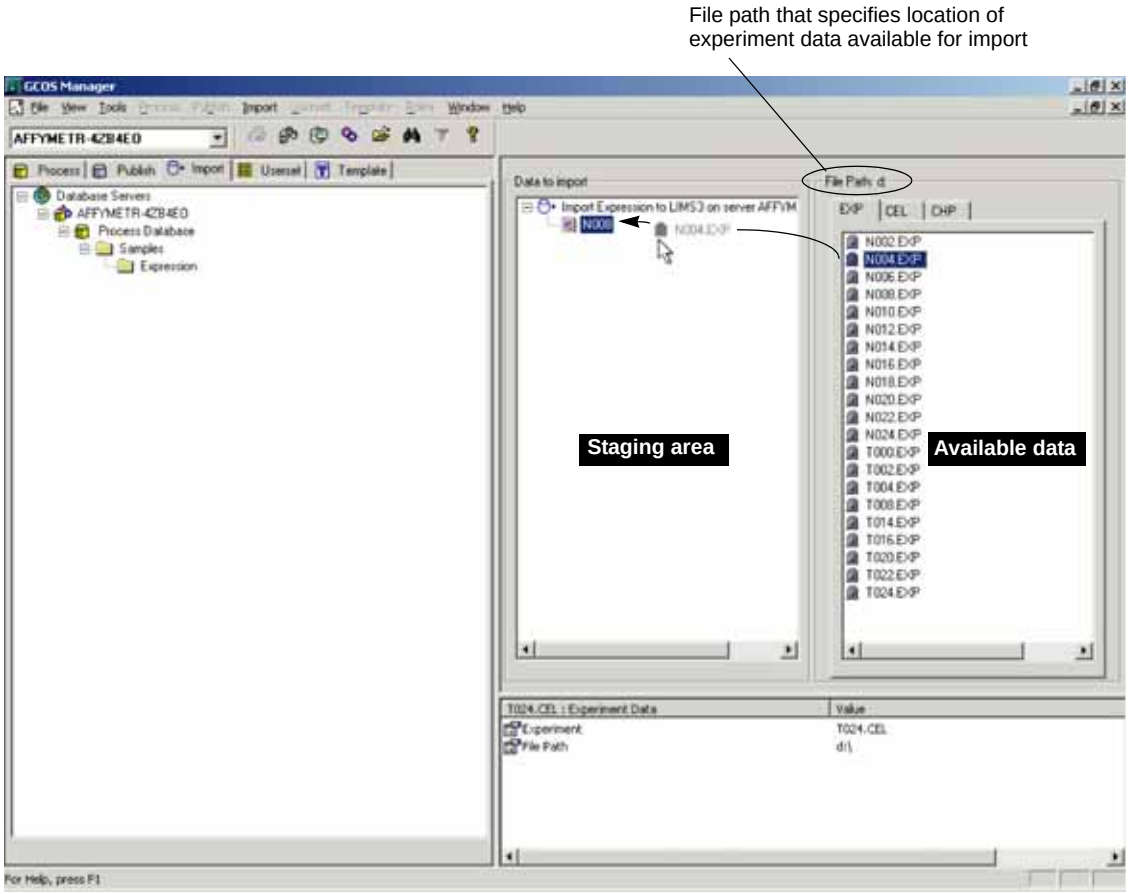


Figure 15.45
Import tab, drag the .exp to associate it with a sample in the staging area

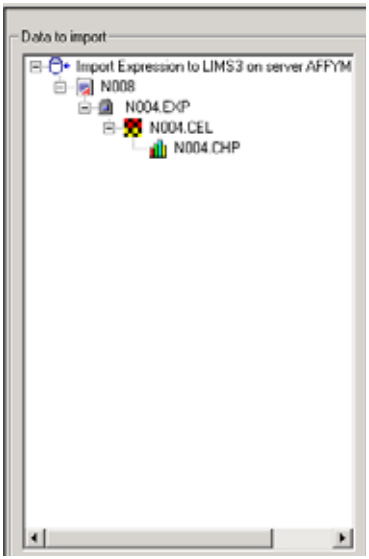


Figure 15.46
Staging Area showing N004.exp and children data associated with sample N008

Associating Additional .cel or .chp with a Sample

You can associate additional .cel or .chp that have different prefixes with a sample that is already associated with an .exp. This is done in a hierarchical manner by adding the .cel to the .exp that is associated with the sample of interest, and adding the .chp to the .cel.

■ To associate a .cel with a sample:

1. Select the CEL tab in the available data pane.
2. Drag the .cel to the .exp that is associated with the sample of interest in the staging area **(Figure 15.47)**. Repeat this step to associate another .cel with the sample.

   ⇒ The .cel is displayed below the .exp **(Figure 15.48)**.

■ To associate a .chp with a sample:

1. Select the CHP tab in the available data pane.
2. Drag the .chp to the .cel that is associated with the sample of interest in the staging area. Repeat this step to associate another .cel with the sample.

   ⇒ The .chp is displayed below the .cel **(Figure 15.48)**.

TIP

To avoid having to associate a .cel and .chp with each .exp imported into the process database, simply import the .exp, then regenerate the.cel and .chp in GCOS using batch analysis. This minimizes potential errors from dragging a .chp to the wrong .cel. See Batch Analysis on page 229 *for more information.*

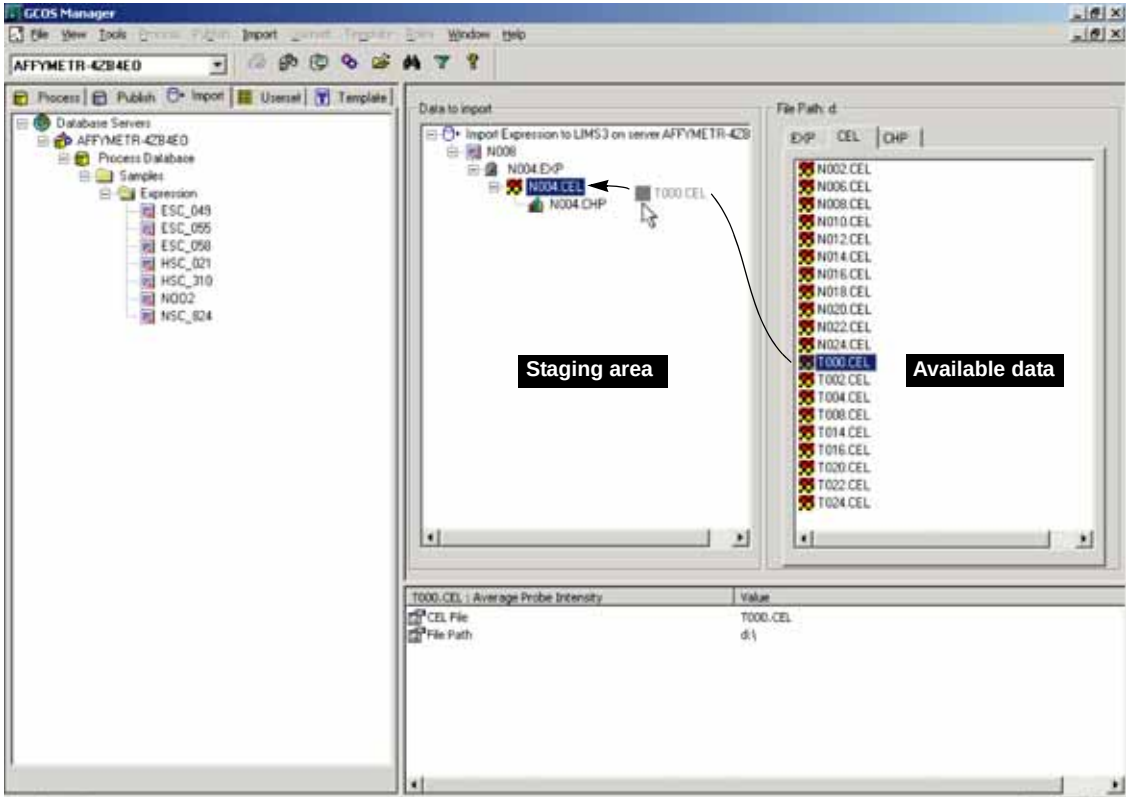


Figure 15.47
Import tab

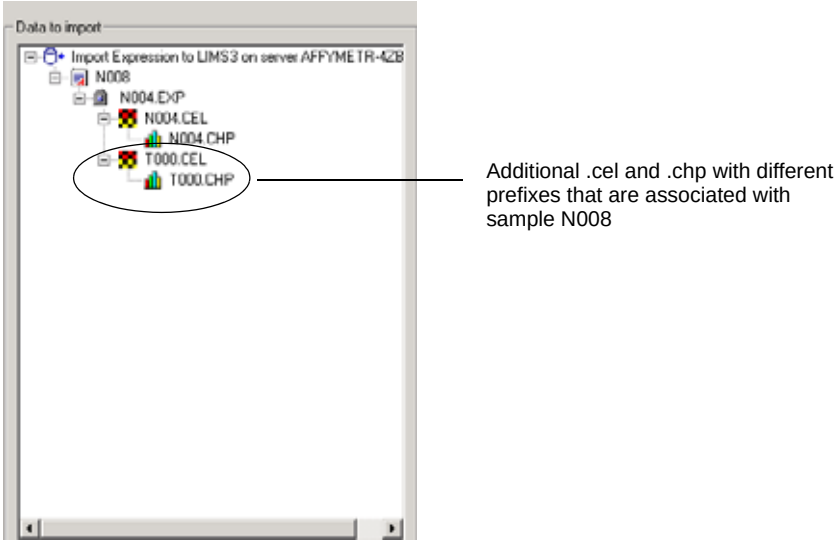


Figure 15.48
Staging area showing .cel and .chp data associated with the sample N008

Removing Data from the Staging Area

You can remove experiment data (.exp, .cel, or .chp) from the staging area. Use either of the following methods to remove data:

- Right-click the sample that you want to remove, and select **Remove** (for data without children) or **Remove All** (for data and associated children data) from the shortcut menu that appears **(Figure 15.43)**. Alternatively, click the sample and select **Import** →**Remove** or **Import** →**Remove All** from the menu bar.

  ⇒ The selected data or data and children data are removed from the staging area.

- Drag the data from the staging area to the available data pane. This must be done in an ascending hierarchical manner by dragging the .chp to the CHP tab, the .cel to the CEL tab, and the .exp to the EXP tab.

Starting the Import

1. Right-click the process database, a sample, or one of the data items and select **Start Import** from the shortcut menu that appears. Alternatively, click one of these items and select **Import →Start Import** from the menu bar.

   ⇒ The import task proceeds and the task status/properties pane displays the import status.

2. To view more information about the import task status, right-click Import in the Task Status/Properties pane and select **View Task Log** from the shortcut menu that appears **(Figure 15.49)**. Alternatively, double-click Import.

   ⇒ The import task log is displayed **(Figure 15.50)**.

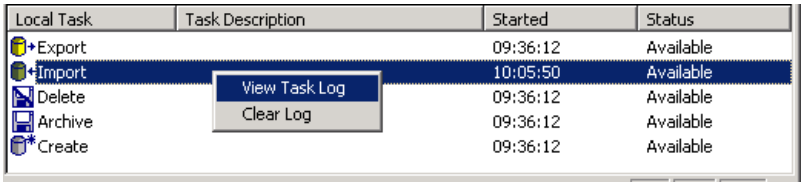


Figure 15.49
Task Status/Properties pane

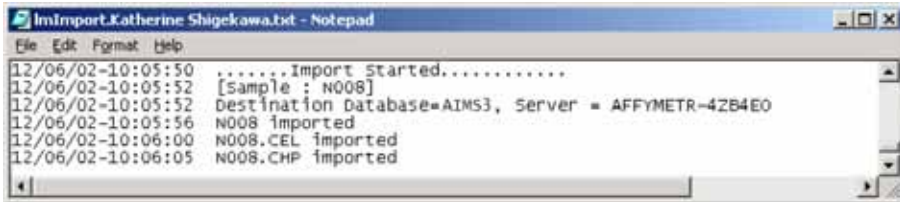


Figure 15.50
Import task log

Userset Tab

This section of the manual explains how to create, modify, or delete a userset. A userset is a set of modifiable Statistical Expression algorithm parameters with predefined values. For more information about the expression algorithm parameters, see *Expression Analysis Algorithm*, on page 515.

A userset is defined by an administrator or other authorized user. It provides a convenient way to select analysis parameters without having to set each parameter individually before an analysis. A userset is applied in GCOS when you analyze expression cell intensity data.

Userset Tab Windowpanes

- Click the Userset tab.

⇒ The three Userset windowpanes are displayed **(Figure 15.38)**.

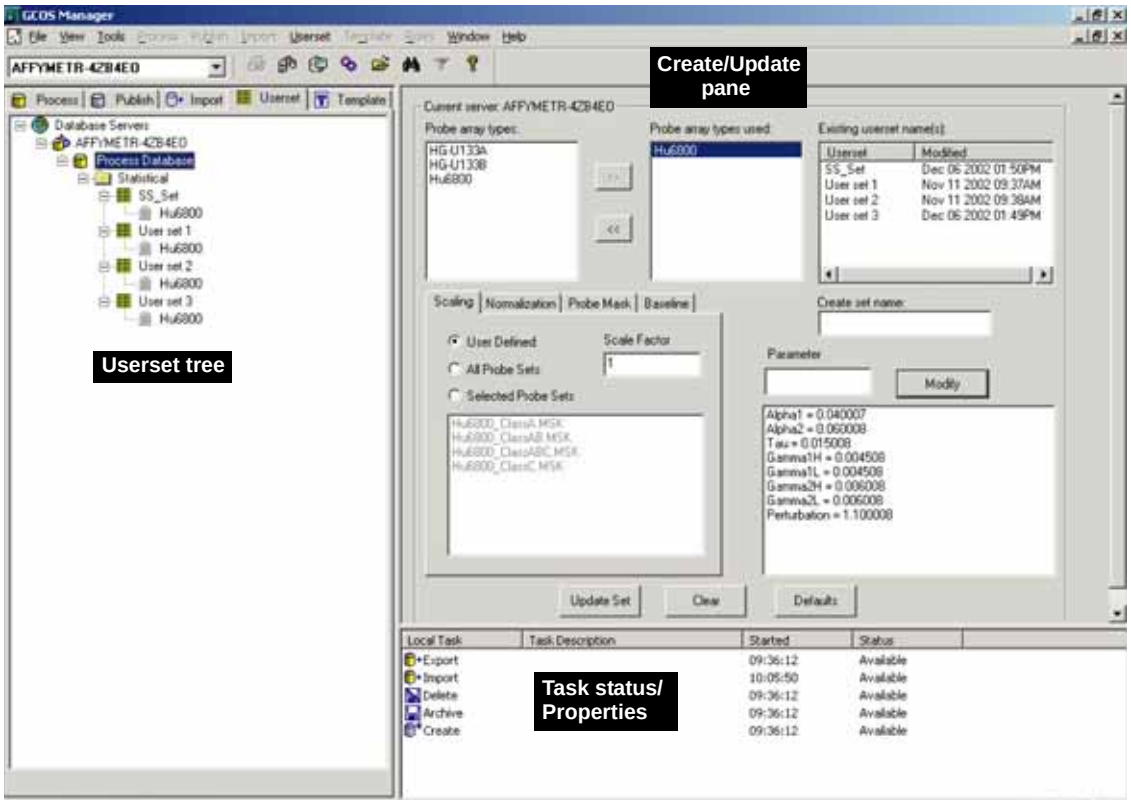


Figure 15.51
Userset tab windowpanes

Userset tree	Shows the active server, process database, user sets and associated probe array types.
Create/ Update pane	Shows new userset information that can be specified or existing userset information that can be modified **(Figure 15.52)**.
Task status/ properties	Displays the property information or local task view, depending on the item selected in process database tree or the selected data pane. Property information is defined by the template that was used during sample registration in GCOS. The task status view shows the status of processes that may be occurring in the database (for example, export, import, delete, archive, or create). In the task status view, you can monitor these jobs while you work on other tasks in the system.

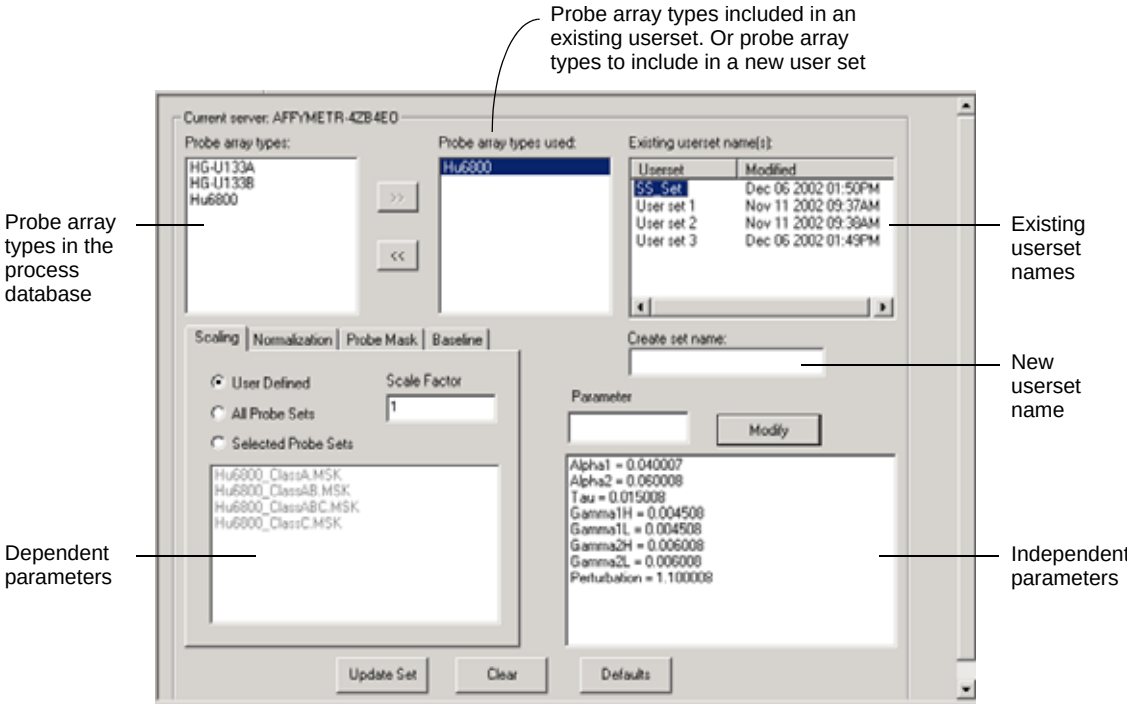


Figure 15.52
Create/Update pane

Creating a Userset

Creating a userset includes the following steps:

- Enter a name for the userset.
- Select the probe array type(s) that the userset will analyze.
- Confirm the defaults or enter new values for the independent parameters.
- Confirm the defaults or enter new values for the dependent parameters.

1. In the **Create set name** box, enter a unique name for the userset.
2. In the **Probe array types** box, select a probe array type and click the arrow button >>. Repeat this step to add other probe array types for the userset.

   ⇒ The selected probe array type(s) is displayed in the **Probe array types used** box and the independent and dependent parameters are set to the default values **(Figure 15.53)**.

3. If you want to use the independent and dependent parameter defaults, click **Create Set**.

   ⇒ The userset is created and the name appears in the **Existing userset name(s)** box.

   If you do not want to accept the defaults, see the following sections on modifying the independent and dependent parameters.

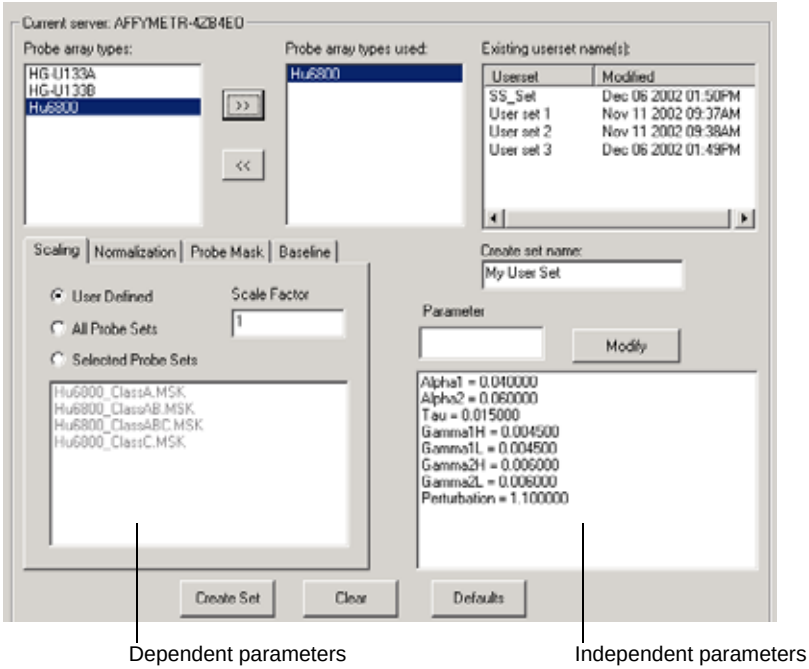


Figure 15.53
Create/Update pane

Modifying Independent or Dependent Parameter Values

The statistical expression algorithm parameter settings are independent parameters. These are static settings that are not influenced by the probe array type.

A dependent parameter is a setting that is specific to a particular probe array type. Dependent parameters include the scaling, normalization, probe mask, and baseline settings. For more information on these parameters, see *Expression Analysis Settings*, on page 532.

1. To modify an independent parameter:

   a. In the independent parameters box, click the parameter that you want to modify.

      ⇒ The parameter name and current setting are displayed in the box above **(Figure 15.54)**.

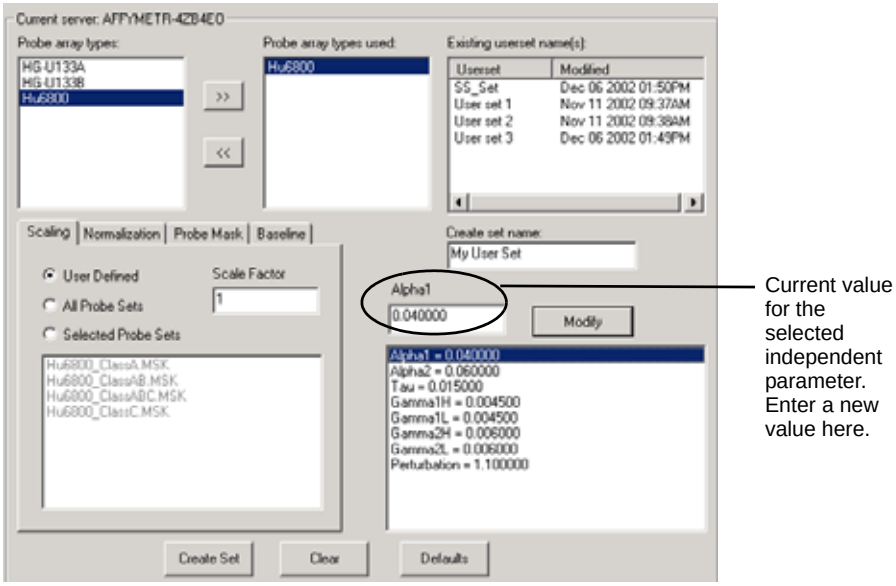


Figure 15.54
Create/Update pane

   b. Enter a new value in the box and click **Modify**.

      ⇒ The new parameter value is displayed in the box below.

c. Repeat step b to modify other independent parameters.

2. To modify a dependent parameter:

   a. Click the tab for the parameter you want to modify (Scaling, Normalization, Probe Mask, or Baseline).

   b. Choose the analysis option that you want to include in the userset and enter any additional necessary information.

3. Click **Create Set** after you finish modifying the parameter values.

   ⇒ The userset is created and the name appears in the **Existing userset name(s)** box **(Figure 15.54)**.

Note *The new parameter values are applied after you click **Create Set**.*

Modifying a Userset

You can modify the independent or dependent parameter values for a userset, or add or remove a probe array type(s) from a userset.

1. In the **Existing userset name(s)** box, click the userset that you want to modify.

   ⇒ The Create/Update pane shows the parameter values for the selected userset **(Figure 15.55)**.

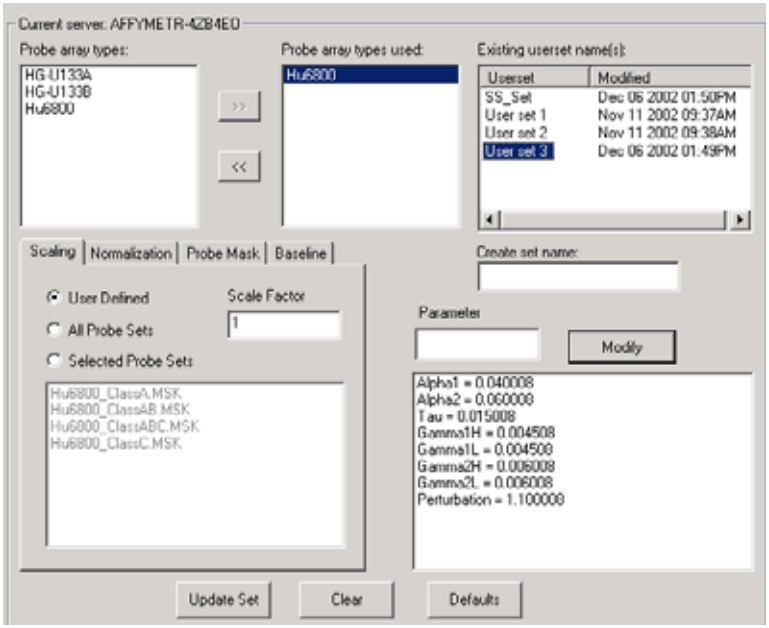


Figure 15.55
Create/Update pane

2. To add a probe array type to the userset, select a probe array type in the **Probe array types** box, and click the arrow button >>. Repeat this step to add other probe array types for the userset.

   ⇒ The selected probe array type(s) is displayed in the **Probe array types used** box **(Figure 15.55)**.

3. To remove a probe array type from the userset, do either of the following:

   - Select a probe array type in the **Probe array types used** box, and click the arrow button <<.

     ⇒ The selected probe array type is removed from the **Probe array types used** box.

   - In the userset tree, expand the Statistical folder and the userset that you want to modify. Right-click the probe array of interest and select **Delete** from the shortcut menu that appears **(Figure 15.56)**.

Note

If you remove a probe array type from the process database in the Process tab, the probe array is also removed from any userset that includes this array type in its Probe Array Types Used list.

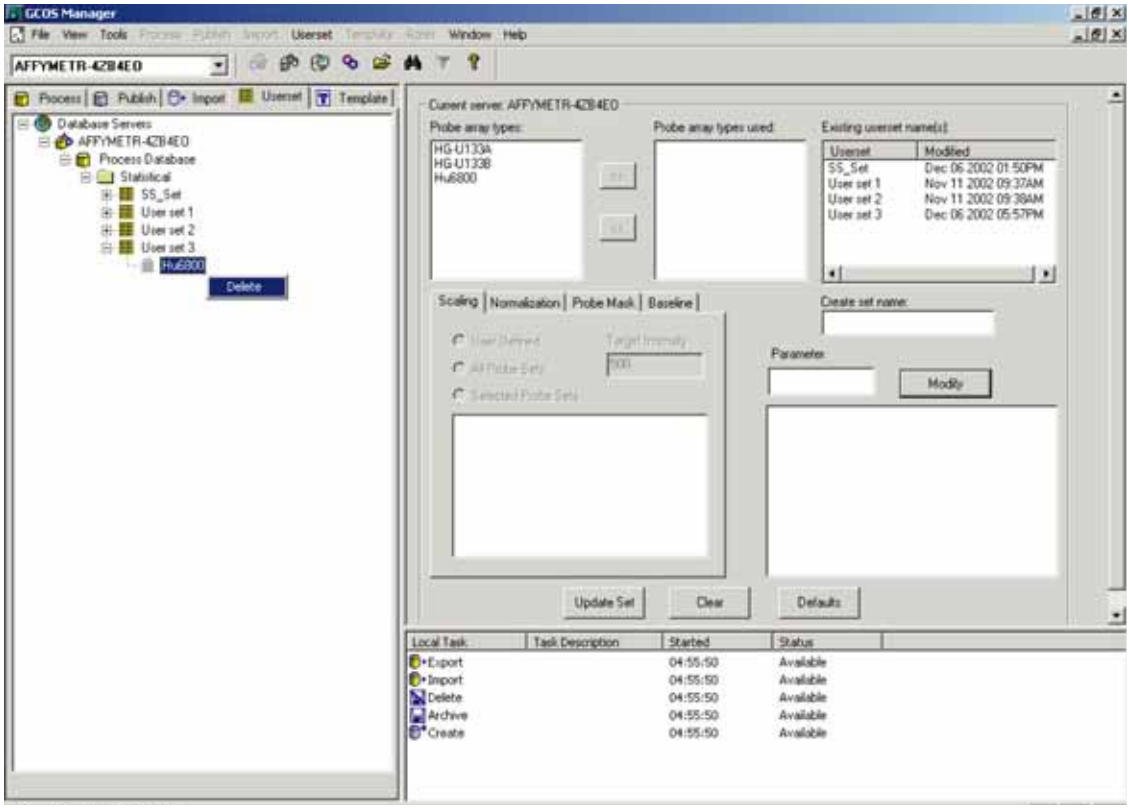


Figure 15.56
Userset tab window

4. To modify an independent or dependent parameter value, see the section *Modifying Independent or Dependent Parameter Values*, on page 339.
5. Click **Update Set** after you finish modifying the userset.
   ⇒ The modifications are saved to the userset and the task status/properties pane displays the Statistical Expression algorithm properties **(Figure 15.57)**.

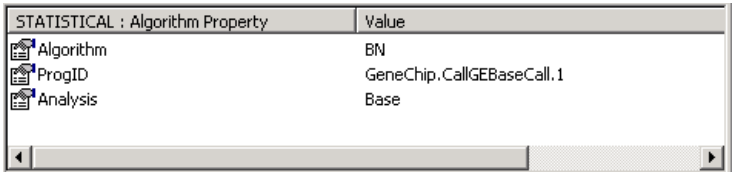


Figure 15.57
Task Status/Properties pane

This information is metadata that exists in the process database and is used internally by GCOS and the GCOS server to distinguish different algorithms in the system.

Deleting a Userset

1. In the userset tree, right-click the userset that you want to delete and select **Delete** from the shortcut menu that appears. Alternatively, click the userset and select **Userset →Delete** from the menu bar.

   ⇒ A warning/confirmation box appears **(Figure 15.58)**.

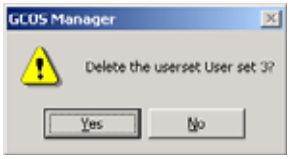


Figure 15.58
Delete userset warning/confirmation box

2. Click **Yes** to delete the userset.

   ⇒ The userset is removed from the userset tree and the **Existing userset name(s)** box in the create/update pane.

Template Tab

A template is a form that defines the attributes and values needed to register a sample or set up an experiment in GCOS. A sample or experiment template is created by the administrator or other authorized user. This section of the manual explains how to create and manage sample or experiment templates.

Template Tab Windowpanes

- Click the Template tab.

  ⇒ The three Template tab windowpanes are displayed **(Figure 15.59)**.

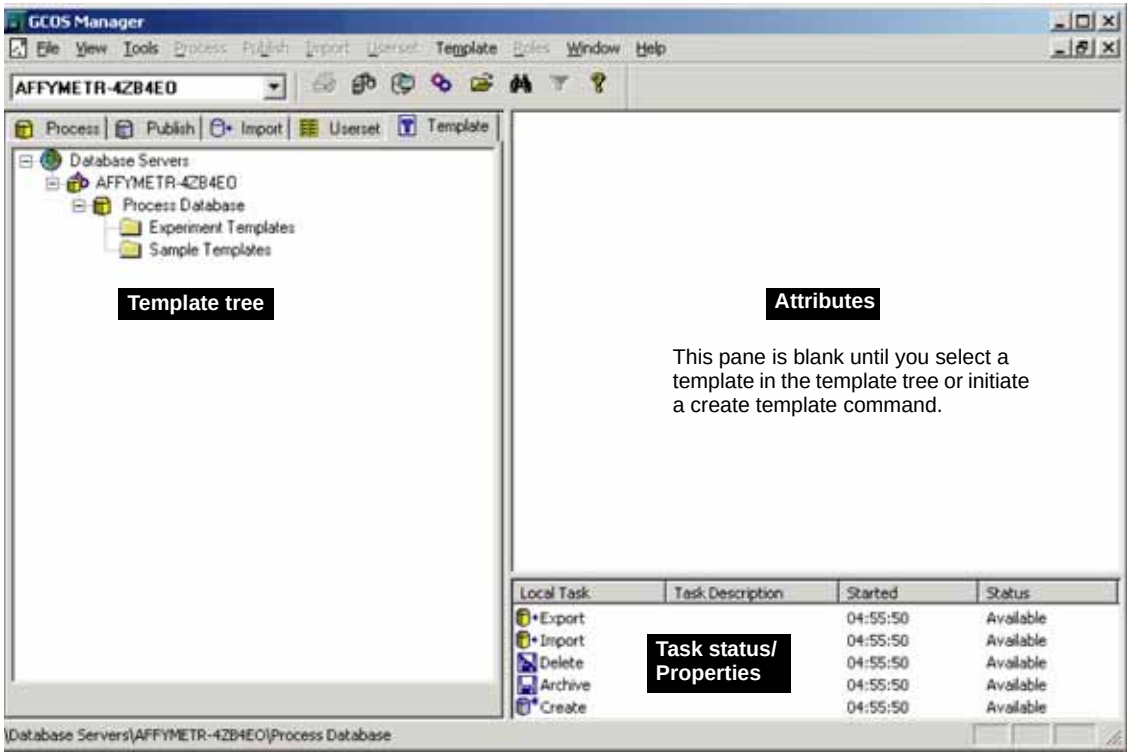


Figure 15.59
Template tab windowpanes

Template tree	Shows the active server, process database, and the experiment and sample templates that reside there. Click a sample or project to display the associated experiments and data (.dat, .cel, .chp) in the data pane. Select a template to display the attributes and values in the attribute pane.
Attributes	Displays the template selected in the template tree. Displays a blank template when a **Create Template** command is initiated.
Task status/ Properties	Displays the property information or local task view, depending on the item selected in process database tree or the selected data pane. Property information is defined by the template that was used during sample registration in GCOS. The task status view shows the status of processes that may be occurring in the database (for example, export, import, delete, archive, or create). In the task status view, you can monitor these jobs while you work on other tasks in the system.

MIAME Sample Template

GCOS Manager provides a sample template based on the Minimum Information About a Microarray Experiment (MIAME).[1] The MIAME sample template includes 23 types of sample attributes. For each attribute, you may:

- define the data type
- specify whether the attribute information is required for sample registration
- specify the values available for a controlled value attribute

1. To view the MIAME Sample Information template, click the **MIAME Sample Information** template in the Sample Templates folder **(Figure 15.60)**.

   ⇒ The Attributes pane displays the MIAME Sample Information template.

2. To enter template information, follow step 5 to step 12 in the section *Creating a Template*, on page 347.

1. A. Brazma, P. Hingamp, J. Quackenbush, G. Sherlock, P. Spellman, C. Stoeckert, J. Aach, W. Ansorge, C.A. Ball, H.C. Causton, T. Gaasterland, P. Glenisson, F.C. P. Holstege, I.F. Kim, V. Markowitz, J.C. Matese, H. Parkinson, A. Robinson, U. Sarkans, S. Schulze-Kremer, J. Stewart, R Taylor, J. Vilo, and M. Vingron. Minimum information about a microarray experiment (MIAME)- toward standards for microarray data. Nature Genetics, 29:365-371.

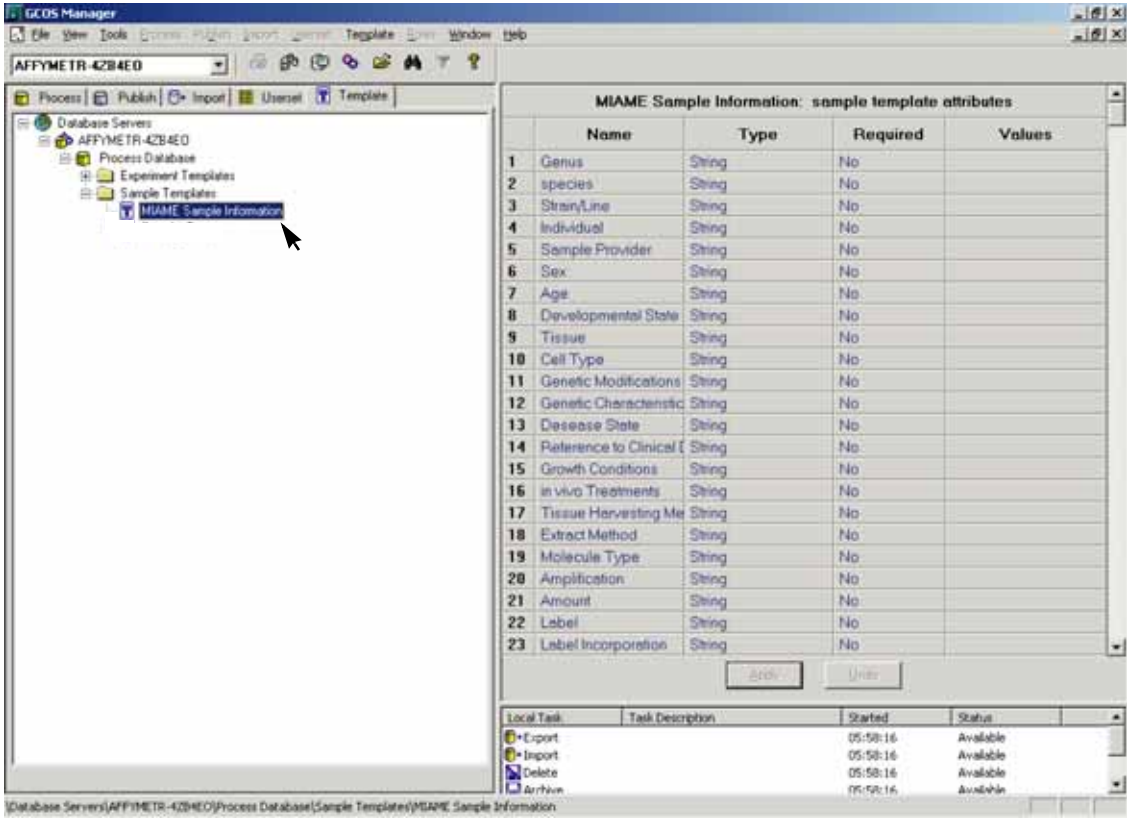


Figure 15.60
MIAME Sample Information template

Working with Templates

Creating a Template

When you create a template, you specify:

- the attributes included in the template
- the data type for each attribute (for example, an integer or a character string)
- whether the attribute is required
- value options for a controlled data attribute

1. To create an experiment template, right-click the Experiment Templates folder and select **Create** from the shortcut menu that appears. Alternatively, click the folder and select **Template →Create** from the menu bar **(Figure 15.61)**.

   To create a sample template, right-click the Sample Templates folder and select **Create** from the shortcut menu that appears. Alternatively, click the folder and select **Template →Create** from the menu bar.

   ⇒ The Create Template box appears **(Figure 15.62)**.

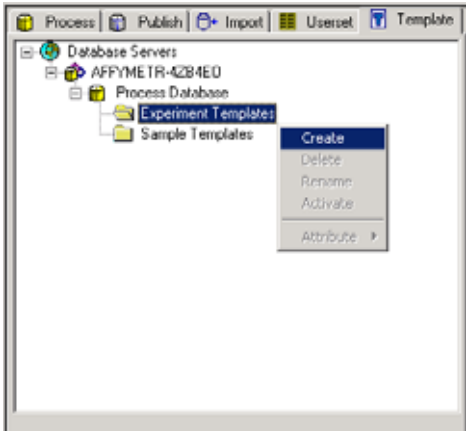


Figure 15.61
Template tree, shortcut menu for experiment templates

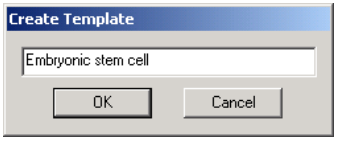


Figure 15.62
Create Template box

2. Enter a name for the template.

   ⇒ A blank template appears in the attribute pane **(Figure 15.63)** and the template name is added to the appropriate folder (experiment or sample). Click the folder and expand it to view the newly added template name.

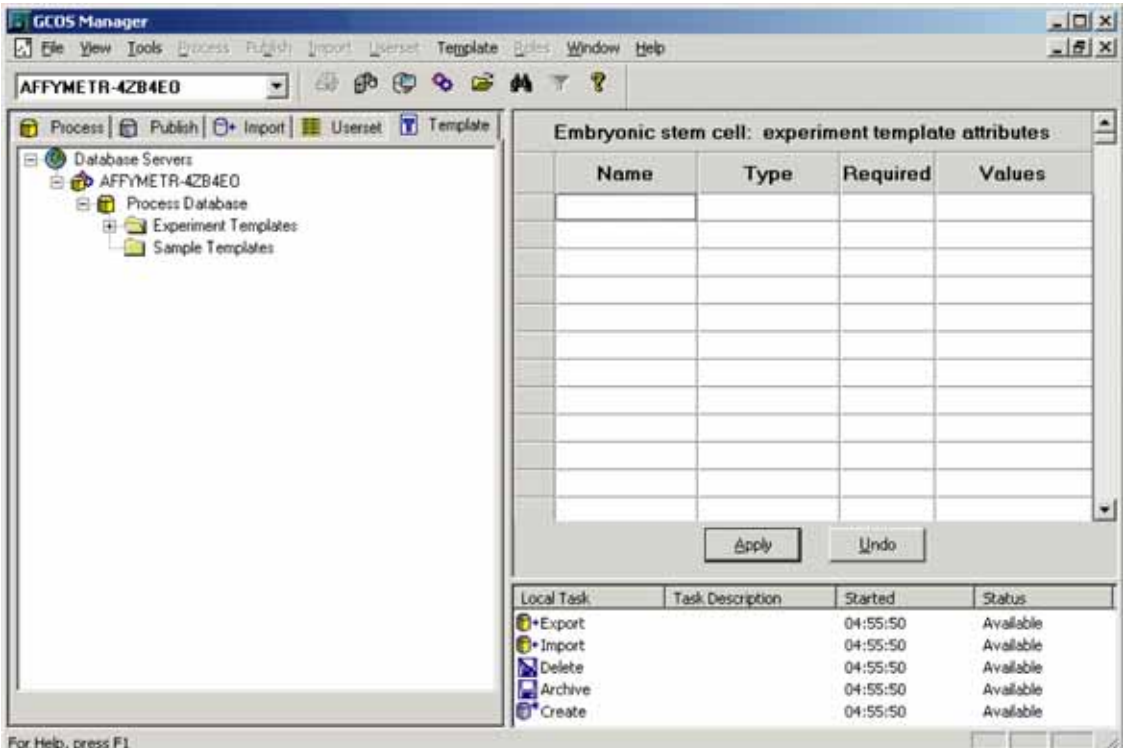


Figure 15.63
Template tab, experiment template

3. In the template, double-click the first blank cell under the Name column.

   ⇒ The mouse pointer becomes an I-beam and the other cells in the row are blocked **(Figure 15.64)**.

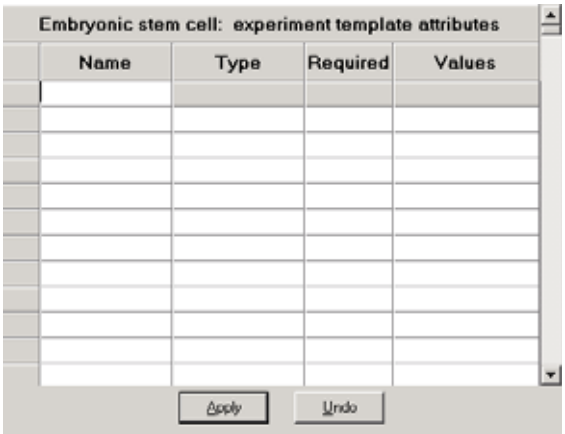


Figure 15.64
Attributes pane

4. Enter an attribute name and press **ENTER**.

   ⇒ The attribute name is inserted and numbered **(Figure 15.65)**.

✔ Note

*During the process of defining an attribute, you can click **Undo** to remove the entries from the Attributes pane.*

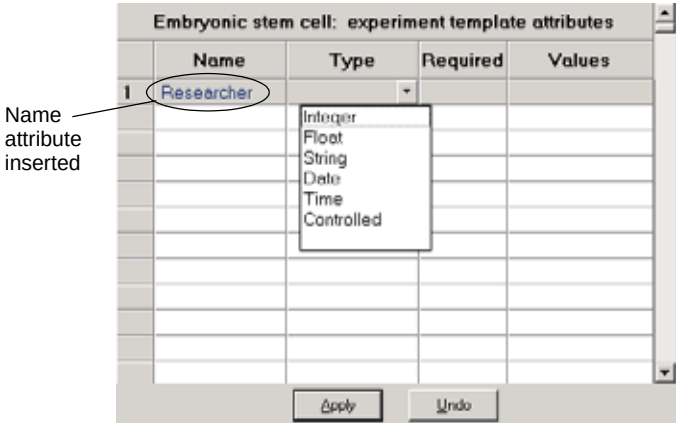


Figure 15.65
Attributes pane

5. Click the cell under the Type column and click the drop-down arrow that appears.

   ⇒ A drop-down list of data type options for the attribute appears **(Figure 15.65)**.

Integer	Whole number
Float	Floating point number
String	Limited to 255 characters
Date	Date
Time	Time
Controlled	Limits user response to the value options listed in the template.

6. Make a selection from the Type drop-down list.

   ⇒ The selected data type is displayed in the cell **(Figure 15.66)**.

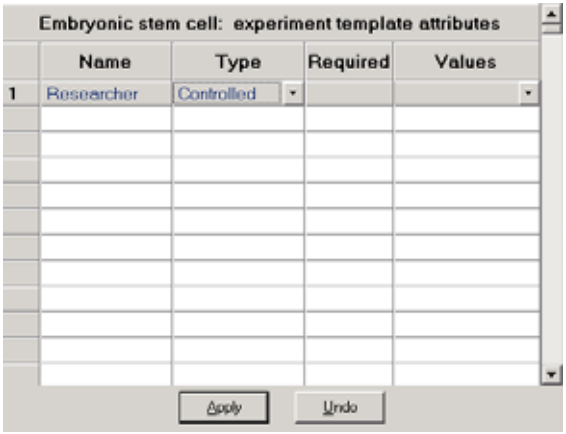


Figure 15.66
Attributes pane

7. Click the cell under the Required column and click the drop-down arrow that appears.

8. In the drop-down list that appears, click **Yes** if the information is required or **No** if it is not **(Figure 15.67)**.

   If the information is required, GCOS requires the user to enter a value during the sample registration or experiment set up process.

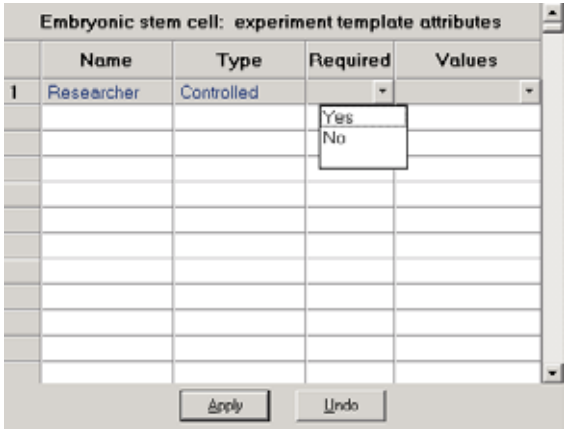


Figure 15.67
Attributes pane

9. If the attribute data type is controlled, double-click the cell under the Values column.

   ⇒ The Controlled Values Edit List box appears **(Figure 15.68)**.

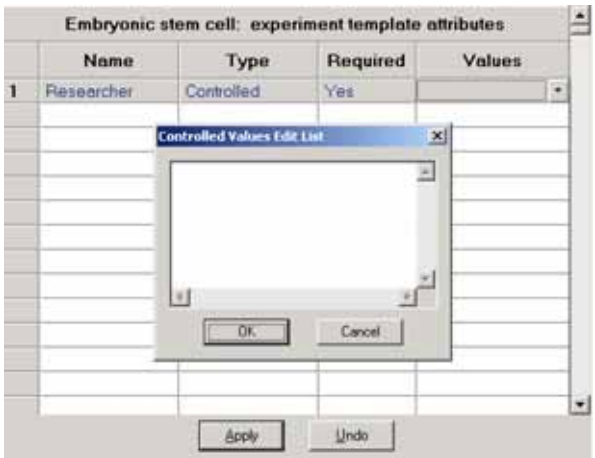


Figure 15.68
Attribute pane and the Controlled Values Edit List box

10. To define a controlled value:
    a. Enter a value option in the box.
    b. Press **Enter**.
    c. Repeat steps a and b to enter additional controlled values.
    d. Click **OK** when finished.
       ⇒ The controlled values appear in the Values drop-down list **(Figure 15.69)**.

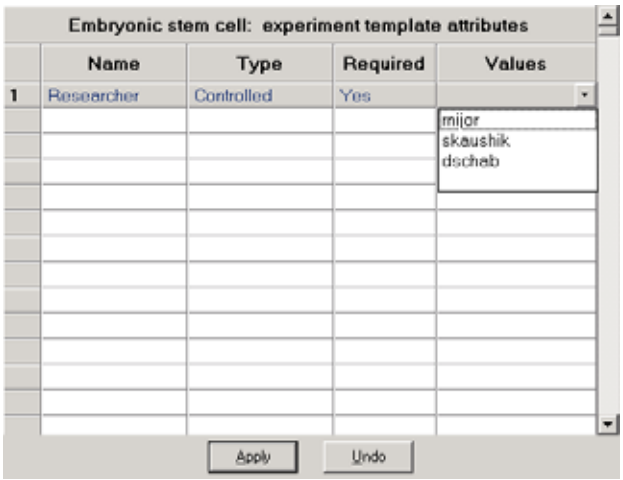


Figure 15.69
Attributes pane and the drop-down list of controlled values for the attribute

11. Click **Apply**.
    ⇒ The defined attributes are now in effect.
12. To define additional attributes for the template, repeat step3 through step 11.

Editing a Template

You can edit an existing template.

1. In the template tree, click the template you want to edit.
   ⇒ The attributes pane displays the selected template.

2. To edit an attribute name, double-click the current entry and enter a new name.
3. Follow step 3 to step 11 in the section *Creating a Template*, on page 347 to edit the attribute definition.

Deactivating or Activating a Template

You can deactivate a template to make it unavailable in GCOS. An inactive template can be activated to restore it for use in GCOS.

1. To deactivate a template:
   a. Right-click the template of interest in the template tree and select **Deactivate** from the shortcut menu that appears. Alternatively, click the template and select **Template →Deactivate** from the menu bar.
   b. Click **OK** in the warning window that appears.
      ⇒ The template icon appears gray in the template tree to indicate the template is inactive and is not available to GCOS.
2. To activate a template:
   a. Right-click the template of interest in the template tree and select **Activate** from the shortcut menu that appears. Alternatively, click the template and select **Template →Activate** from the menu bar.
      ⇒ The template icon is restored to full color in the template tree to indicate the template is active and is available to GCOS.

Renaming a Template

1. In the template tree, right-click the template that you want to rename and select **Rename** from the shortcut menu that appears. Alternatively, click the template and select **Template →Rename** from the menu bar.
2. Enter a name for the template and press **ENTER**.

✔ Note *You cannot duplicate an existing name for the same type of template.*

Working with Attributes

To view a shortcut menu of attribute commands, right-click an attribute number **(Figure 15.70)**. Alternatively, click an attribute number and select **Template →Attribute** from the shortcut menu that appears.

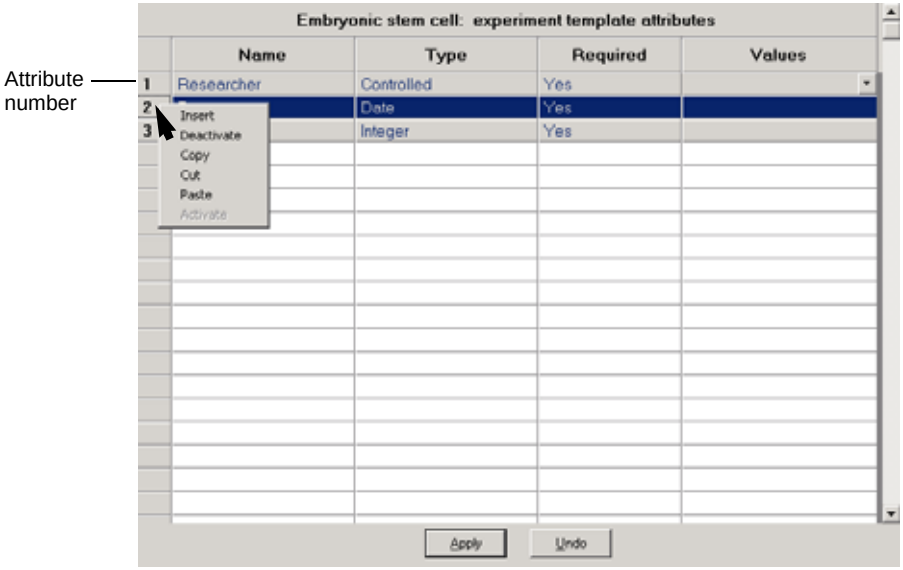


Figure 15.70
Attributes pane, shortcut menu

- To select an attribute for a command, click the attribute number.
- To select a group of adjacent attributes, press and hold the **Shift** key while you click the number of the first and last attribute in the group.
- To select non-adjacent attributes, press and hold the **Ctrl** key while you click the attribute numbers.

Inserting an Attribute

You can insert a blank row in the template.

1. Right-click the attribute number below the insertion point and select **Insert** from the shortcut menu that appears. Alternatively, click the attribute number and select **Template →Insert** from the menu bar.

   ⇒ A blank row is inserted in the template.

2. Define the attribute and click **Apply**.

Deactivating an Attribute

A deactivated attribute is not displayed in the template for sample registration or experiment setup in GCOS.

1. Right-click the number of the attribute that you want to deactivate and select **Deactivate** from the shortcut menu that appears. Alternatively, click the attribute number and select **Template →Attribute → Deactivate** from the menu bar.

   ⇒ The attribute remains in the template, but appears grayed-out.

2. Click **Apply**.

   ⇒ A warning message appears.

3. Click **OK** to deactivate the attribute.

✔ Note

In GCOS, the attribute is displayed in samples and experiments that were saved using the attribute. These cannot be modified. A deactivated attribute is not available for new samples or experiments.

Activating an Attribute

1. Right-click the number of the attribute that you want to activate and select **Activate** from the shortcut menu that appears. Alternatively, click the attribute number and select **Template →Attribute →Activate** from the menu bar.

   ⇒ The attribute is no longer grayed-out.

2. Click **Apply**.

   ⇒ The attribute becomes active and is available in the template.

Cutting, Copying or Pasting an Attribute

1. To cut an attribute, right-click the attribute number and select **Cut** from the shortcut menu that appears. Alternatively, click the attribute number and select **Template →Attribute →Cut** from the menu bar.

   ⇒ The attribute information is copied to the system clipboard.

2. To copy an attribute, right-click the attribute number and select **Copy** from the shortcut menu that appears. Alternatively, click the attribute number and select **Template →Attribute →Copy** from the menu bar.

   ⇒ The attribute information is copied to the system clipboard.

3. To paste an attribute, right-click the attribute number and select **Paste** from the shortcut menu that appears. Alternatively, click the attribute number and select **Template →Attribute →Paste** from the menu bar.

   ⇒ The attribute information on the system clipboard is pasted on the template at the end of the attribute list.

4. Click **Apply**.

Roles Tab

✔ Note

The Roles tab is only available to GCOS users connected to a GCOS server.

A role is a defined set of user access privileges. The administrator or other authorized user can create roles with different levels of access privilege for different users or groups of users. In this way, the roles function provides data security. This section of the manual explains how to create and manage roles.

Roles Tab Window

- Click the Roles tab to view the three Roles tab windowpanes **(Figure 15.71)**.

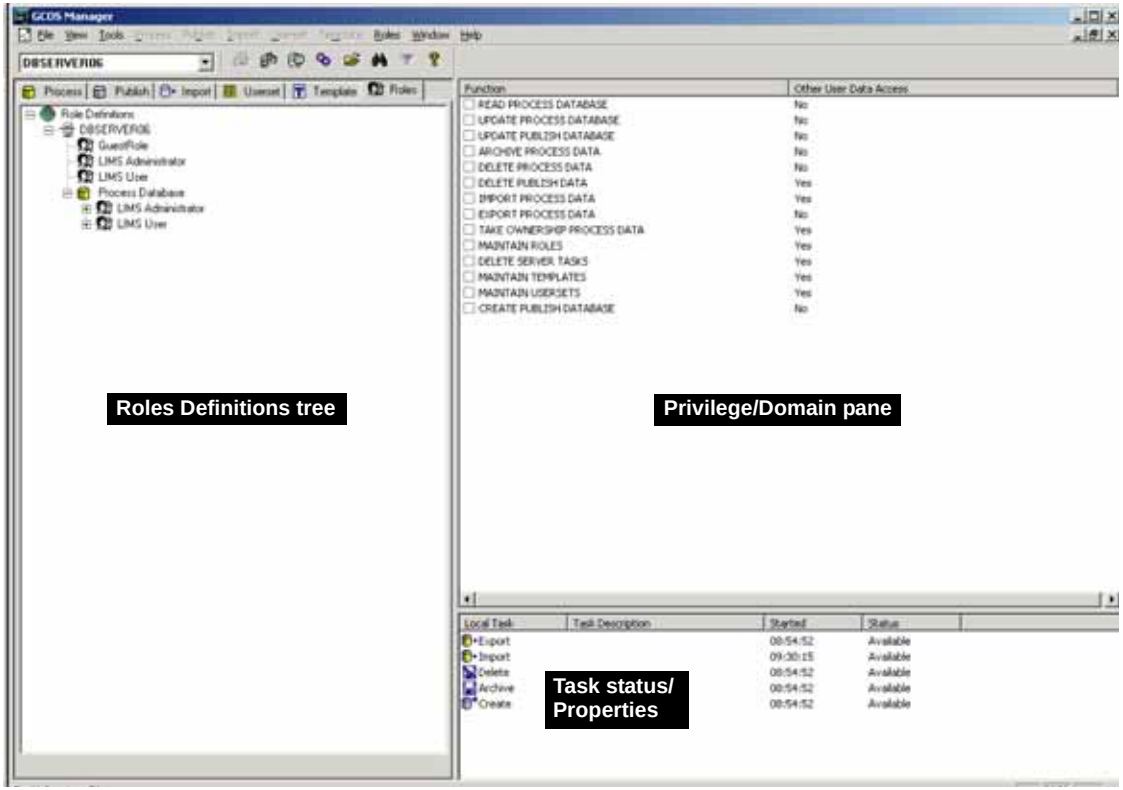


Figure 15.71
Roles tab windowpanes

Role Definitions tree	Displays the active server, the process database, the roles created on the server, and the members of each role **(Figure 15.72)**.
Privileges pane	Displays a list of the domains that reside on the active server. Shows the privileges that are associated with a role selected in the role definitions tree. Displays a template of privileges that is used to define privileges for a new role.
Task status/ Properties	Displays the property information or local task view, depending on the item selected in process database tree or the selected data pane. Property information is defined by the template that was used during sample registration in GCOS. The task status view shows the status of processes that may be occurring in the database (for example, export, import, delete, archive, or create). In the task status view, you can monitor these jobs while you work on other tasks in the system.

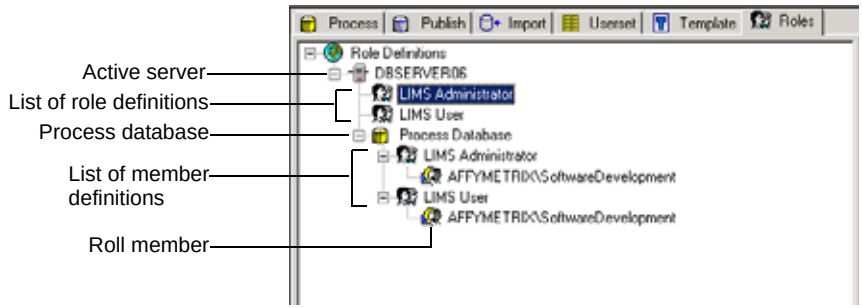


Figure 15.72
Role definitions tree

Creating a Role

A role defines a set of user access privileges. Each GCOS user is assigned to a role. Creating a role is a three-step process:

- Name the role.
- Define the role privileges (what the user can and cannot do).
- Define role membership (add users to the role).

Naming the Role

1. Right-click an existing role in the role definitions tree and select **Create Role** from the shortcut menu that appears. Alternatively, click the role and select **Role →Create Role** from the menu bar.

   ⇒ The Create Role box appears **(Figure 15.73)**.

Figure 15.73
Create Role box

2. Enter a name for the role and click **OK**.

   ⇒ The role name appears in the role definitions tree **(Figure 15.74)**.

Note *The role name is required and cannot duplicate an existing name.*

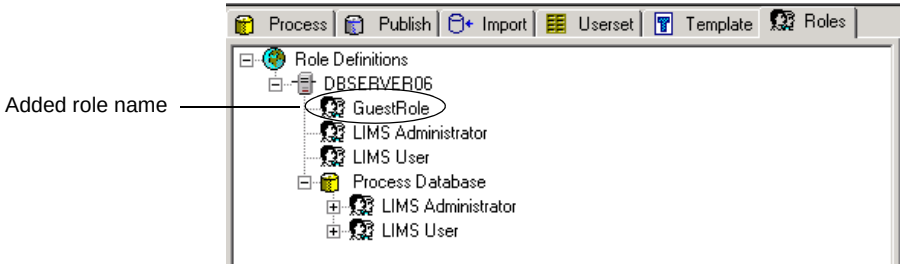


Figure 15.74
Role definitions tree

3. In the role definitions tree, drag the role name to the process database.

   ⇒ The role is added to the list of member definitions under the process database.

Figure 15.75
Drag the role to the process database

Defining Privileges

1. In the role definitions tree, click the role that you want to define.

   ⇒ The Privileges pane displays a template of rights and privileges **(Figure 15.76)**.

Function	Other User Data Access
☐ READ PROCESS DATABASE	No
☐ UPDATE PROCESS DATABASE	No
☐ UPDATE PUBLISH DATABASE	No
☐ ARCHIVE PROCESS DATA	No
☐ DELETE PROCESS DATA	No
☐ DELETE PUBLISH DATA	Yes
☐ IMPORT PROCESS DATA	Yes
☐ EXPORT PROCESS DATA	No
☐ TAKE OWNERSHIP PROCESS DATA	Yes
☐ MAINTAIN ROLES	Yes
☐ DELETE SERVER TASKS	Yes
☐ MAINTAIN TEMPLATES	Yes
☐ MAINTAIN USERSETS	Yes
☐ CREATE PUBLISH DATABASE	No

Figure 15.76
Default privilege settings

In the privilege template, the function list shows the privileges that can be granted to a role member with respect to their own data. The second column called Other User Data Access defines the rights of the role member with respect to other users' data. **Table 15.3** defines the privilege functions.

2. Click the check box next to a function to select that privilege for the role with respect to the user's own data.

3. Click **Yes** /**No** in the right column to toggle between the two options and define the user privilege with respect to other users' data.

Note

*Other User Data Access is always **Yes** and cannot be toggled for the following functions: Take Ownership, Maintain Roles, Import Process Data, and Delete Publish Data.*

Table 15.13
Role privileges

Function	Allows a user to...
Read Process Database	Browse sample, experiment or analyses information in the process database.
Update Process Database	Modify sample and experiment properties information in the process database.
Update Publish Database	Overwrite experiment and analyses information in the publish database.
Archive Process Data	Archive .dat, .cel, or .chp data to an user-specified location.
Delete Process Data	Delete sample, experiment, analyses, userset and probe array type information from the process database.
Delete Publish Data	Delete experiment and analyses information from any publish database.
Import Process Data	Migrate Affymetrix® GCOS data files into the process database.
Export Process Data	Copy sample, experiment, and analysis data from the GCOS server to a defined location.
Take Ownership of Process Data	Assume ownership of a sample, experiment or analysis information with the ability to overwrite a file with new contents (for example, reanalyze a .cel with different expression analysis parameter settings).
Maintain Roles	Manage role, role membership, and set privileges.
Delete Server Tasks	Delete publish tasks on or before a given date.
Maintain Templates	Manage templates and their attributes.
Maintain Usersets	Manage expression analysis parameter sets.
Create Publish Database	Create a publish database.

Defining Role Membership

You add a user to a role in order to assign the user the privileges that are defined for the role. You can also remove a user from a role.

Adding a User to a Role

1. In the role definitions tree, click the role of interest in the list of member definitions (under the process database).

   ⇒ The privilege/domain pane displays the domain name tree **(Figure 15.77)**.

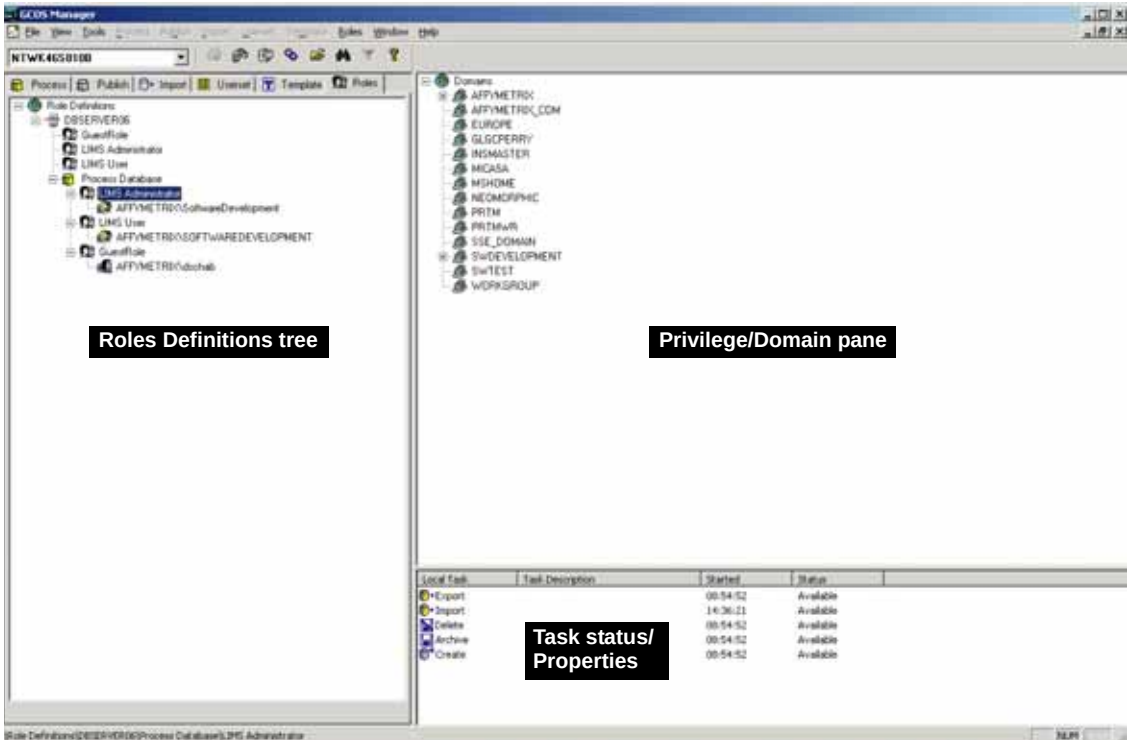


Figure 15.77
Roles tab, domain names

2. Expand the domain names and locate the user name of interest.

   ⇒ The tree displays the user groups that reside on the domain.

3. Drag the user name to the role of interest in the member definitions list.
   ⇒ The user is added to the role and the role name displays the new member name **(Figure 15.78)**.

✔ Note *Click and drag the icon to add an entire user group to a role.*

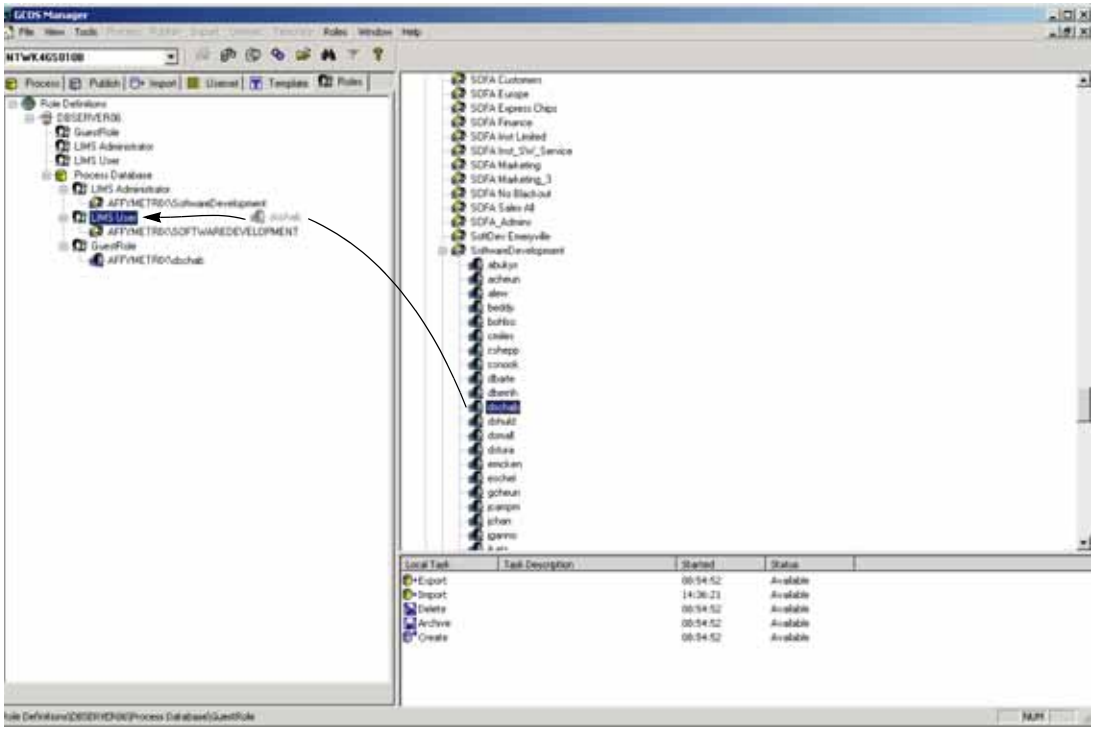


Figure 15.78
Roles tab

Deleting a User from a Role

1. In the role definitions tree (under the process database), expand the user list for the role of interest.
2. Right-click the member that you want to delete and select **Remove User** from the shortcut menu that appears **(Figure 15.79)**. Alternatively, click the user and select **Roles →Remove User** from the menu bar.

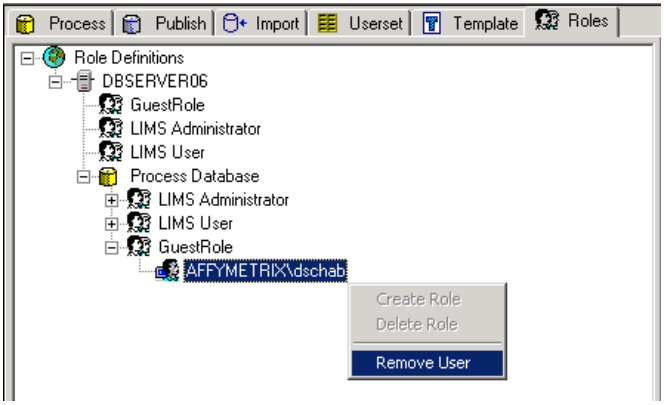


Figure 15.79
Role definitions tree

3. The user name is removed from the role membership.

Deleting Roles

Note

LIMS User and LIMS Administrator are default roles and cannot be deleted.

1. In the role definitions tree (under the active server), right-click the role that you want to delete and select **Delete Role** from the shortcut menu that appears **(Figure 15.80)**. Alternatively, click the role and select **Roles** → **Delete Role** from the menu bar.

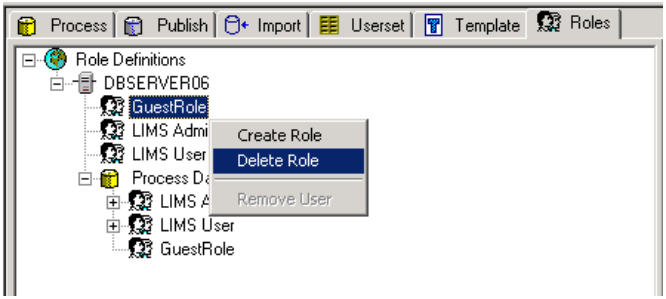
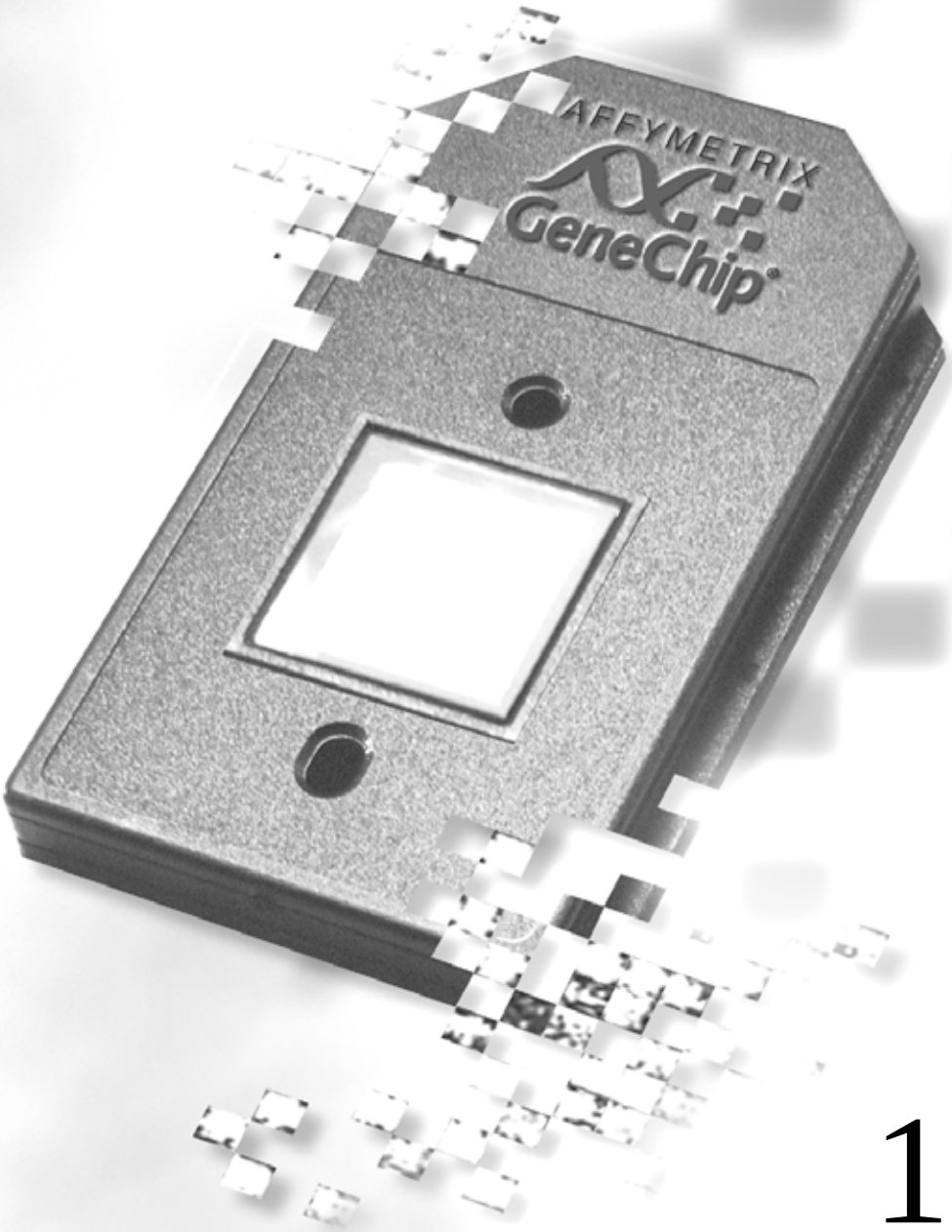


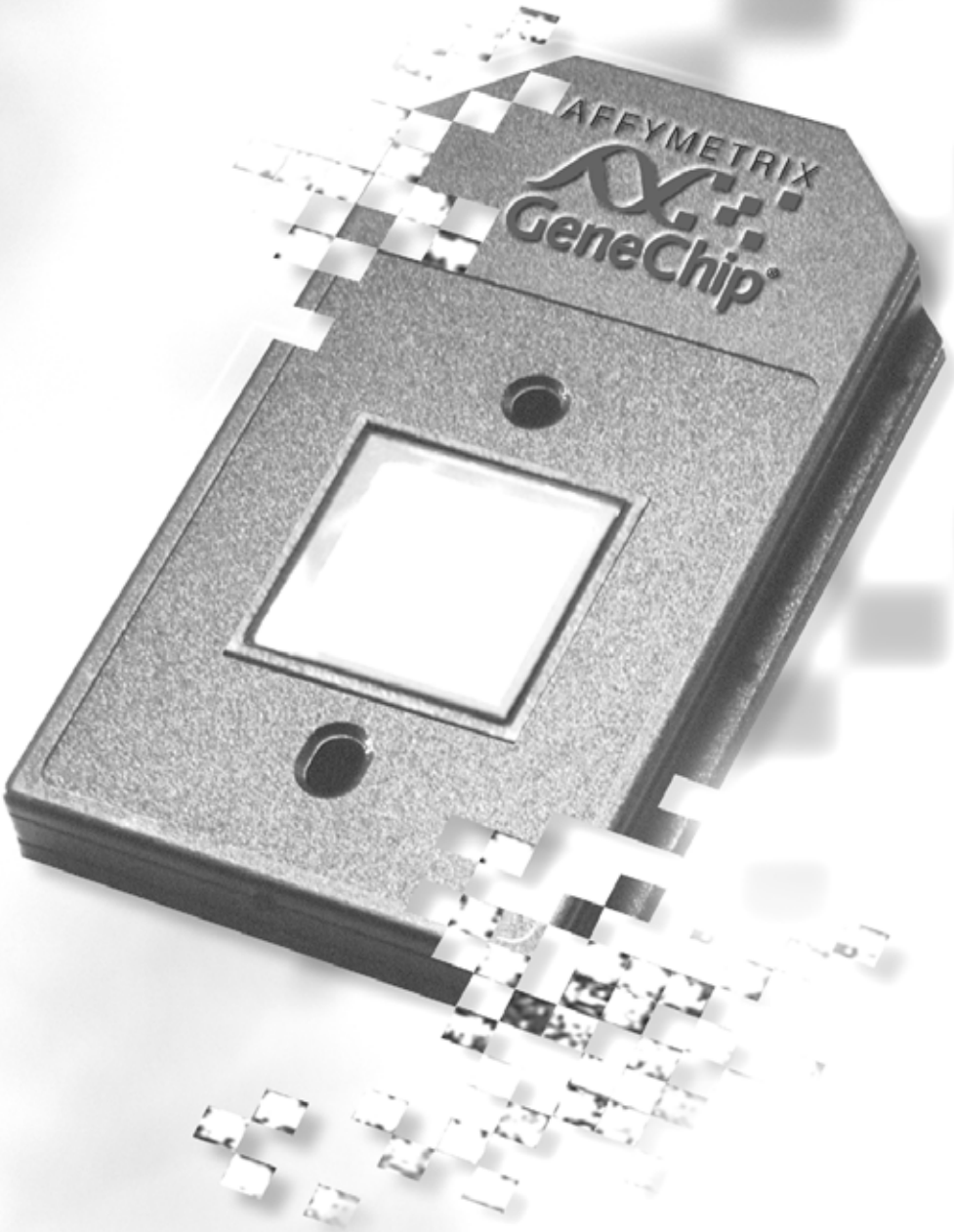
Figure 15.80
Role definitions tree

2. In the warning/confirmation box that appears, click **Yes** to proceed.
   ⇒ The role is deleted and is removed from the role definitions tree.



16

Chapter 16



GCOS Administrator

The GCOS Administrator provides tools to backup a database as well as data and help you manage your workstation disk space. This section of the manual explains how to use the GCOS Administrator to:

- Backup (copy) a database, project, sample, or experiment to a compressed file format (.cab)
- Restore a database or data from a .cab file to a user-selected workstation drive or GCOS server
- Automatically backup the process database on the workstation
- Monitor available space on a workstation drive or database
- Relocate a publish database between workstation drives
- Register or unregister a GCOS server

Getting Started

1. To start the GCOS Administrator, click the Windows **Start** menu button , then select **Programs →Affymetrix →GCOS Administrator**.

   ⇒ The GCOS Administrator starts and displays the user interface.

   Figure 16.1 shows the user interface at startup when the workstation is the active server and **Figure 16.2** shows the user interface at startup when a GCOS server is selected.

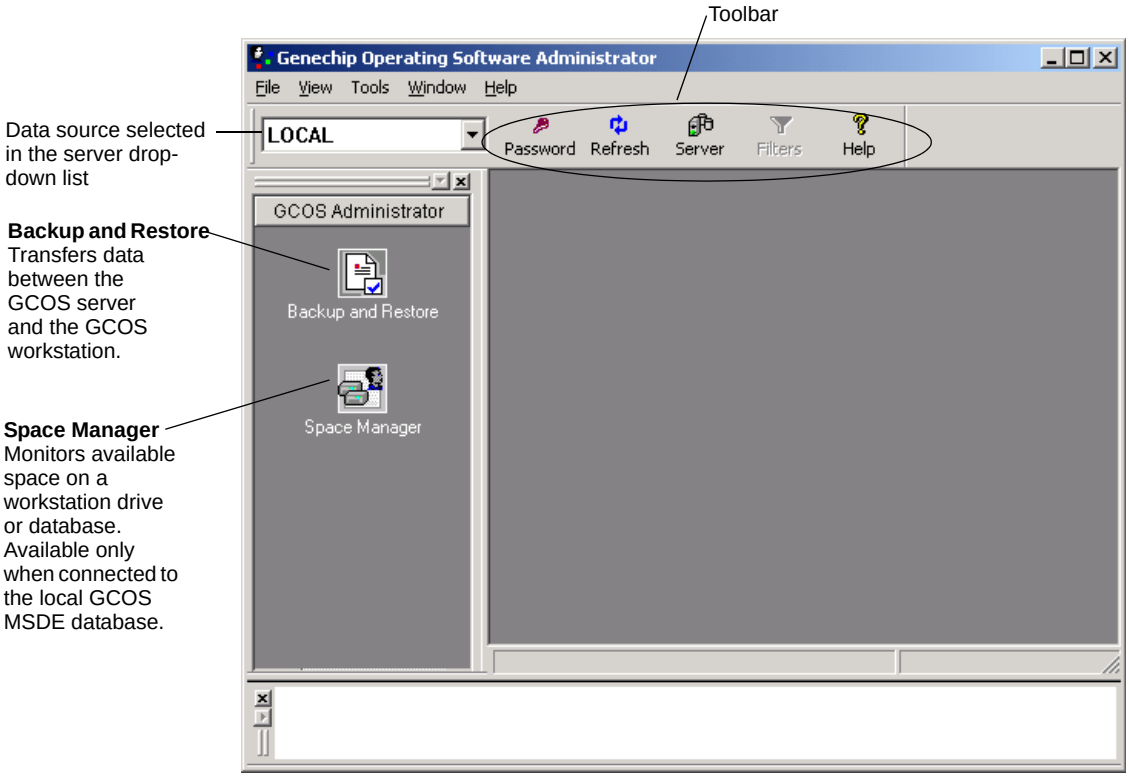


Figure 16.1
GCOS Administrator user interface at startup, workstation server selected

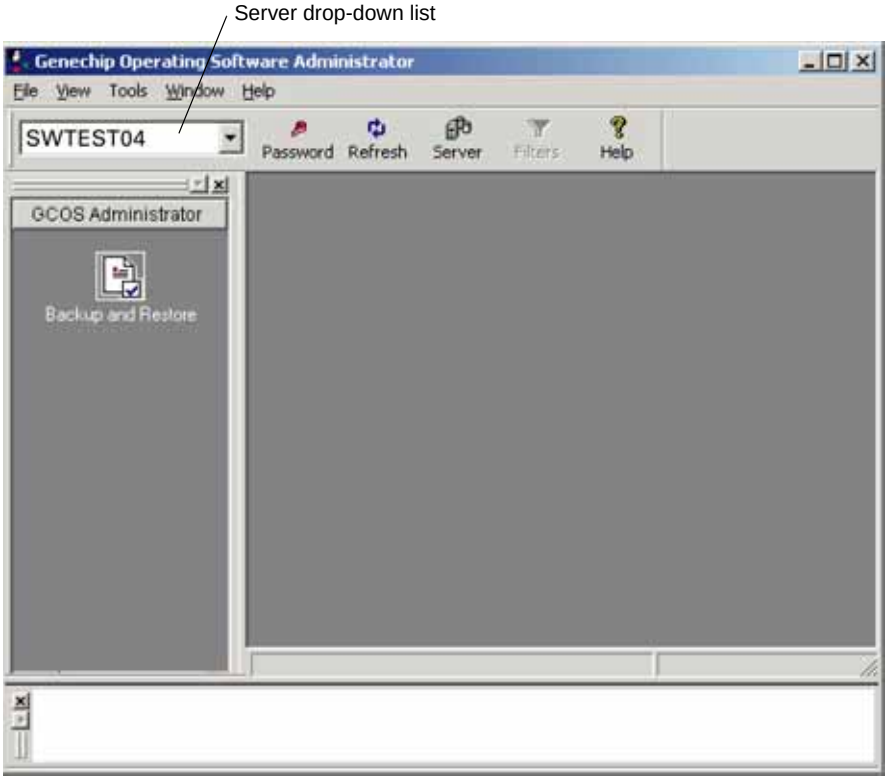


Figure 16.2
GCOS Administrator user interface at startup, GCOS server selected

2. To select the active server, make a selection from the server drop-down list in the toolbar.
3. To display the GCOS Administrator backup and restore window, click the **Backup and Restore** button .
   ⇒ A data tree and property pane are displayed **(Figure 16.3)**.

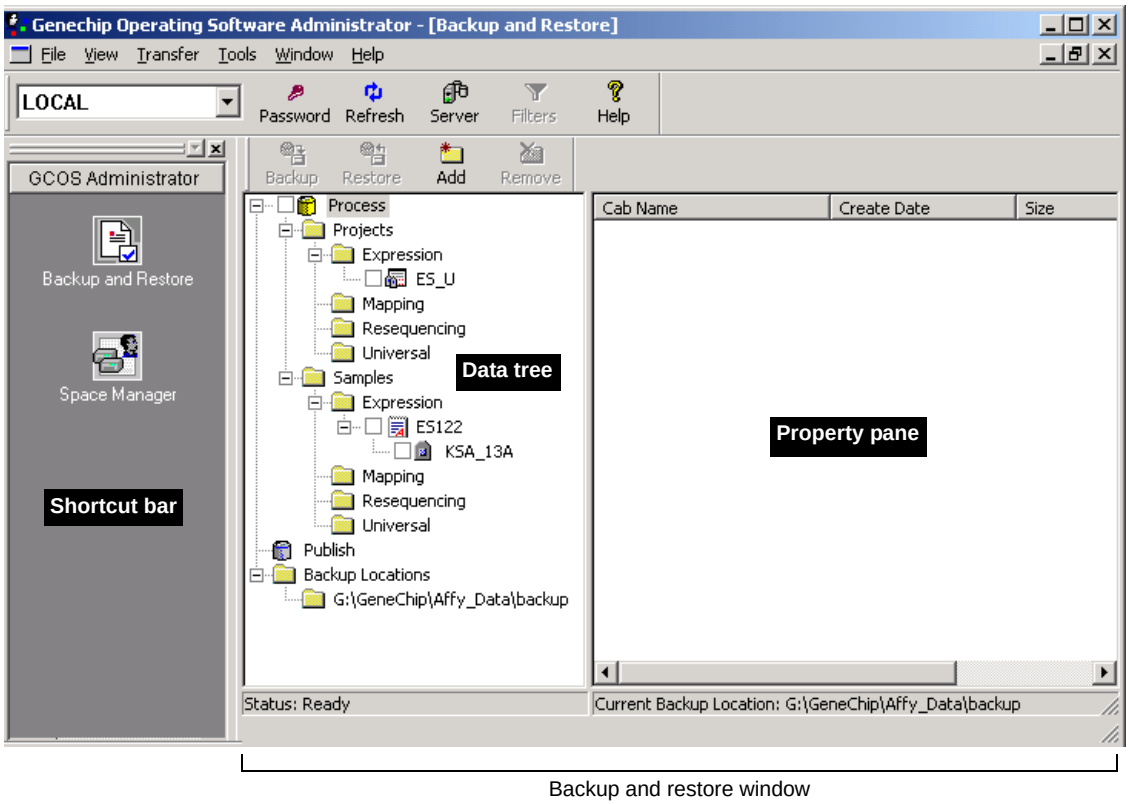
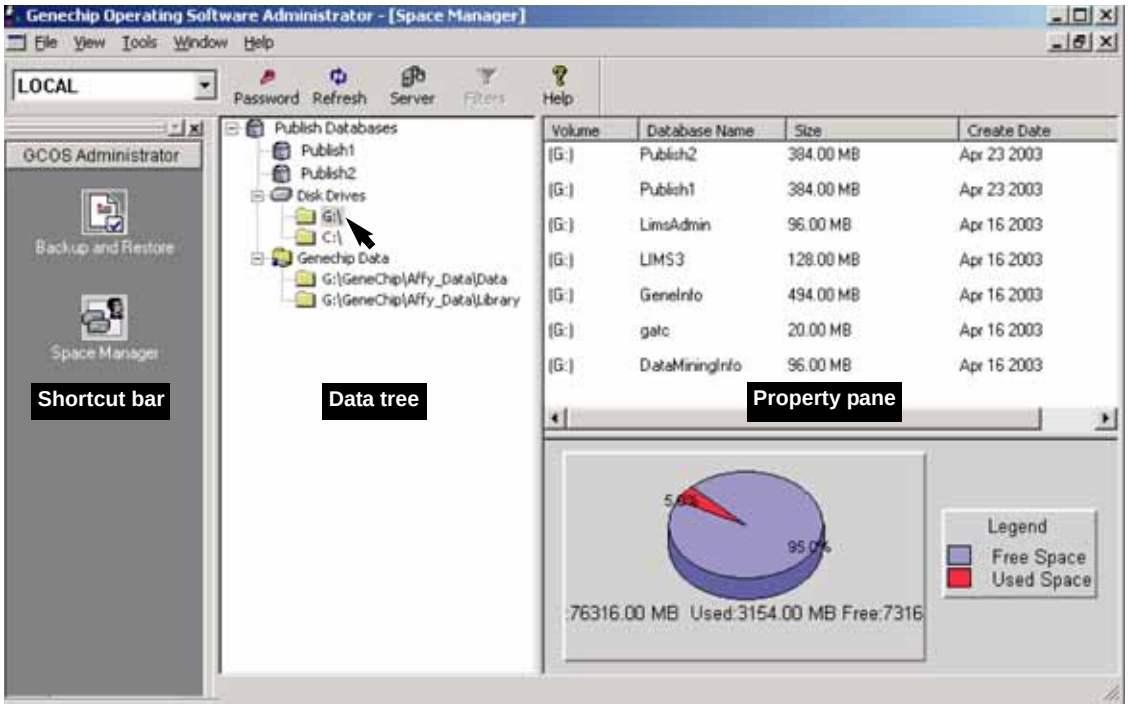


Figure 16.3
GCOS Administrator, backup and restore window

4. To display the Space Manager window:
   a. Select **Local** from the active server drop-down list.
   b. Click the **Space Manager** button .
   ⇒ A data tree and property pane are displayed **(Figure 16.4)**.

✔ Note *The Space Manager functions are only available for the workstation database and not for a GCOS server.*



Space Manager window

Figure 16.4
GCOS Administrator, Space Manager window for the workstation database

GCOS Administrator Shortcut Bar & Windowpanes

Shortcut bar Click the **Backup and Restore** button to display the backup and restore window. Click the **Space Manager** button to display the Space Manager window for the workstation database.

Data tree Backup and restore window: displays the process and publish databases on the active server, the assay projects and samples in the process database, and locations for backup locations.

Space Manager window (for the workstation): displays the publish databases on the active server, workstation drives, and the locations of the probe information (library) files and experiment data. (The file locations are in GCOS.)

Property pane Backup and restore window: displays the cab files at the selected backup location.

Space Manager window (for the workstation): displays property information for the item selected in the data tree, the free and used space for a selected database or drive, or directory size.

Filtering Samples

In the backup and restore window, you can filter the samples that are displayed in the data tree **(Figure 16.5)**. Sample filters are available when you click the Samples directory. The filters remain active from session to session until the parameters are changed.

1. Click the **Backup and Restore** button .
2. In the data tree, click the Samples folder.

   ⇒ The data tree displays the samples and the **Filter** button becomes available.

3. Click the **Filter** button .

   ⇒ The Sample Filter dialog box appears **(Figure 16.5)**.

4. Select filter parameters from the drop-down boxes.

5. Click **Apply**.

   ⇒ The sample filters are applied and the data tree is updated.

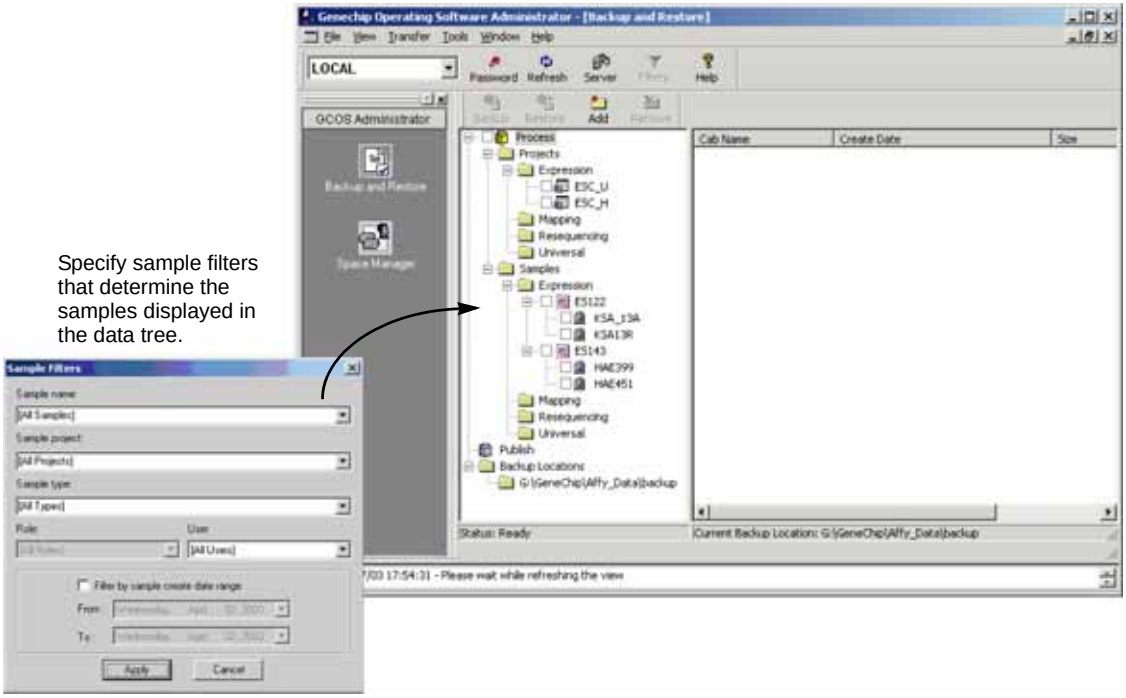


Figure 16.5
Sample filters can be applied to the data tree in the backup and restore window

Role Privileges

Table 16.1 summarizes the role privileges that are required to carry out GCOS Administrator functions on a GCOS server. Privileges can be set using GCOS Manager. For more information on setting role privileges, see *Creating a Role*, on page 359. These privileges are not required for functions carried out on the GCOS workstation.

Table 16.1
Privilege requirements for GCOS Administrator functions on a GCOS server

Function on GCOS Server	Privilege Required on GCOS Server
Backup operation (create a .cab file)	EXPORT PROCESS DATA
Restore a .cab file created by the same user	IMPORT PROCESS DATA
Restore a .cab file created by another user	IMPORT PROCESS DATA with other user data access rights
Copy a .cab file created by the same user to a GCOS server	IMPORT PROCESS DATA
Copy a .cab file created by another user to a GCOS server	IMPORT PROCESS DATA with other user data access rights
Copy a .cab file created by the same user from a GCOS server	EXPORT PROCESS DATA
Copy a .cab file created by another user from a GCOS server	EXPORT PROCESS DATA with other user data access rights
Delete a process database .cab file created by the same user	DELETE PROCESS DATA
Delete a process database .cab file created by another user	DELETE PROCESS DATA with other user data access rights
Delete a publish database .cab file created by the same user	DELETE PUBLISH DATA
Delete a publish database .cab file created by another user	DELETE PUBLISH DATA with other user data access rights

Register or Unregister a GCOS Server

GCOS Administrator can register or unregister a GCOS server. The GCOS server can be the source of objects (a publish database or data) that you want to backup or the destination for objects that you want to restore. This enables the transfer of a publish database or data between a workstation and a GCOS server.

Note

You may have more than one registered GCOS server; however, GCOS, GCOS Manager, and GCOS Administrator can connect to only one GCOS server at a time.

Registering a GCOS Server

1. Click the **Server** button. Alternatively, select **Tools →Register GCOS Server** from the menu bar.

   ⇒ The Register GCOS Server box appears **(Figure 16.6)**.

Figure 16.6
Register GCOS Server box

2. Select the server that you want to register and click **OK**.

   ⇒ The GCOS server name is added to the server drop-down list.

Unregistering a GCOS Server

1. Select the GCOS server that you want to unregister in the server drop-down list **(Figure 16.17)**.

Figure 16.7
Server drop-down list

2. Press the **Delete** key or select **Tools →Unregister GCOS Server** from the menu bar.

   ⇒ The GCOS server is unregistered and the server name is removed from the server drop-down list.

Changing a Database Password

You can change the password for the process database or a publish database on the workstation.

1. Click the **Password** button .

   ⇒ The Administrator Password Dialog opens **(Figure 16.8)**.

Figure 16.8
Administrator Password Dialog

2. Make a selection from the Database drop-down list.
3. Enter the current password for the selected database.
4. Enter a new password for the database.
5. Re-enter the password to confirm it.
6. Click **OK**.

Automatic Process Database Back Up

When the workstation is turned on, the GCOS Administrator automatically starts and runs in the background. The system tray displays the GCOS Administrator icon. For example, **Figure 16.9** shows the GCOS application open and the GCOS Administrator running in the background.

The GCOS Administrator monitors the number of analyses that are added to the process database on the workstation. After every 50 analyses added to the process database, the software automatically backs up the process database to the GCOS directory (\backup subfolder).

Note

The automated backup feature is only available for a process database on the workstation. It is not available for a process database on a GCOS server.

Figure 16.9
GCOS application window is open and GCOS Administrator runs in the background

- To maximize the GCOS Administrator application, double-click the icon.
- To access a shortcut menu for the GCOS Administrator, right-click the GCOS Administrator icon in the system tray.

Shortcut Menu Command	Function
Maximize	Maximizes the GCOS Administrator window.
Minimize	Minimizes the GCOS Administrator window.
Exit	Exit the GCOS Administrator application.

If you exit the GCOS Administrator application, the process database will not be automatically updated.

Copying a Database or Data

The GCOS Administrator can make a backup copy of a process or publish database, a project, a sample, or an experiment. The software copies a database or data to a .cab file, a compressed file format, that is saved to a user-specified backup location. **Table 16.2** lists the items available for back up and the contents of the .cab file.

To perform backup operations on a GCOS server, you must have the EXPORT PROCESS DATA role privilege. If you perform a backup operation and are not in the GCOS Administrator role, each experiment is checked to see whether you have the right to back up the experiment based on your role. For more information on roles and privileges, see *Roles Tab*, on page 357 in Chapter 15. This privilege can be set using GCOS Manager and is not required for back up on the GCOS workstation.

You can conveniently transfer a database or data to another workstation drive or GCOS server by restoring a .cab file to a user-specified location. See *Restoring a CAB File*, on page 390.

Table 16.2
Back up object and CAB file contents

Object Selected for Backup	CAB File Contents
Process database	All of the items in the process database, including projects, samples, experiment and sample annotations, image data, cell intensity data, and probe analysis data.
Publish database	Cell intensity data, probe analysis data.
Project	Samples, image data, cell intensity data, and probe analysis data.
Sample	Sample, image data, cell intensity data, probe analysis data), and all associated annotations.
Experiment	Sample, image data, cell intensity data, probe analysis data), and all associated annotations.

1. To copy a database or data, click the **Backup and Restore** button in the shortcut bar .

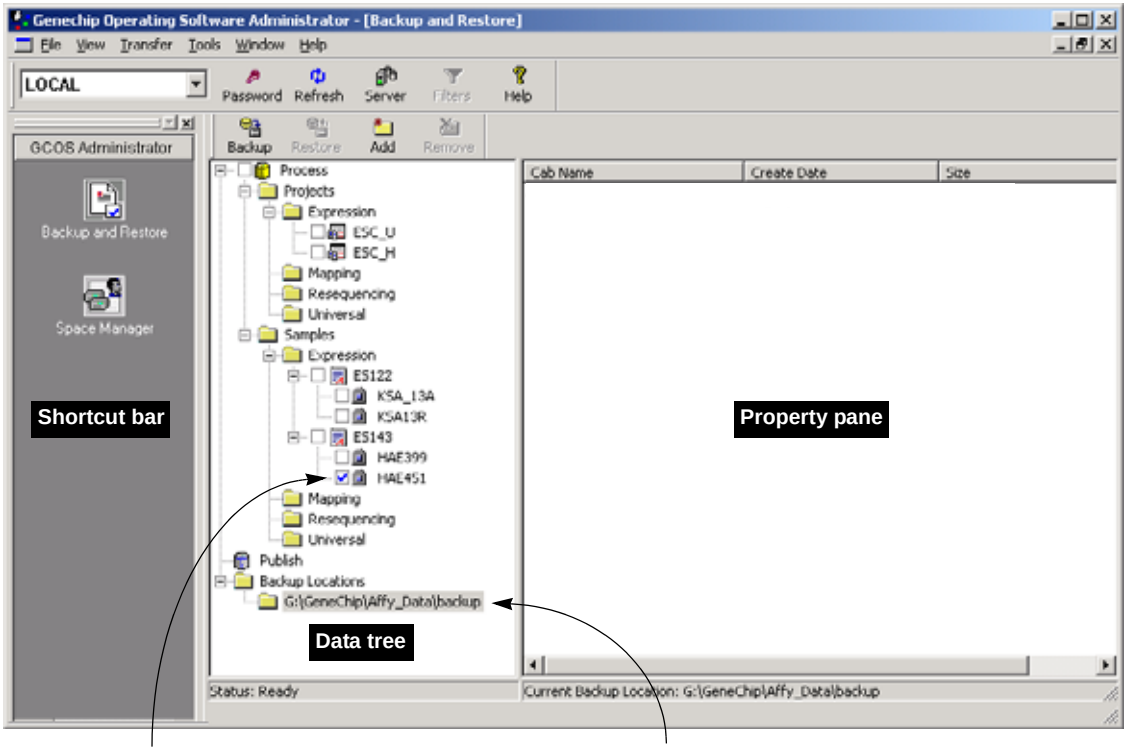


Figure 16.10
GCOS Administrator, backup and restore window

2. Click the check box next to each item that you want to copy to a .cab file **(Figure 16.10)**.

   To remove a check mark, click the check box again.

3. To select a location for the .cab file, navigate to the Backup Locations directory in the data tree and click a backup location.

4. Click the **Backup** button . Alternatively, select **Transfer →Backup** from the menu bar.

   ⇒ The Backup Job Information dialog box appears **(Figure 16.11)**.

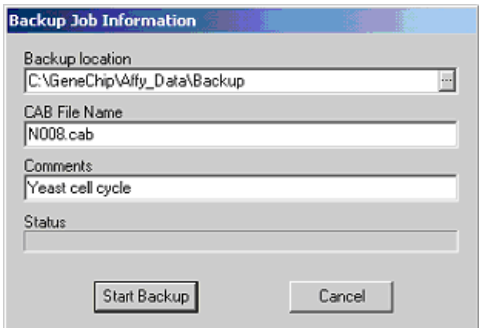


Figure 16.11
Backup Job Information

5. To change the backup location for the .cab:

   a. Click the **Browse** button ...

      ⇒ The Browse for Folder box appears **(Figure 16.12)**.

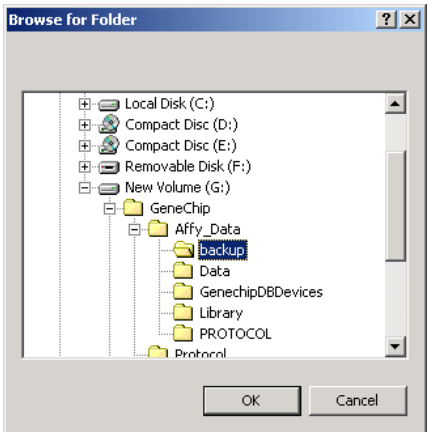


Figure 16.12
Browse for Folder box

b. Click the folder where you want to store the .cab, and click **OK**.

6. Enter the .cab file name (required) and any comments in the Backup Job Information box.

   Unless the user performing the backup operation is in the GCOS Administrator role, each experiment is checked to see whether the user has the right to back up the file. Experiments that are not authorized to be backed up will be rejected and a warning message states that the experiment was not backed up due to authorization.

7. Click **Start Backup** in the Backup Job Information box.

   ⇒ The backup operation starts and the status pane displays messages about its progress. When the backup is completed, the property pane displays the .cab file(s) **(Figure 16.13)**.

✔ Note

You can create multiple copies of a database or data object, but each .cab file must be given a unique name when saving to the same backup location.

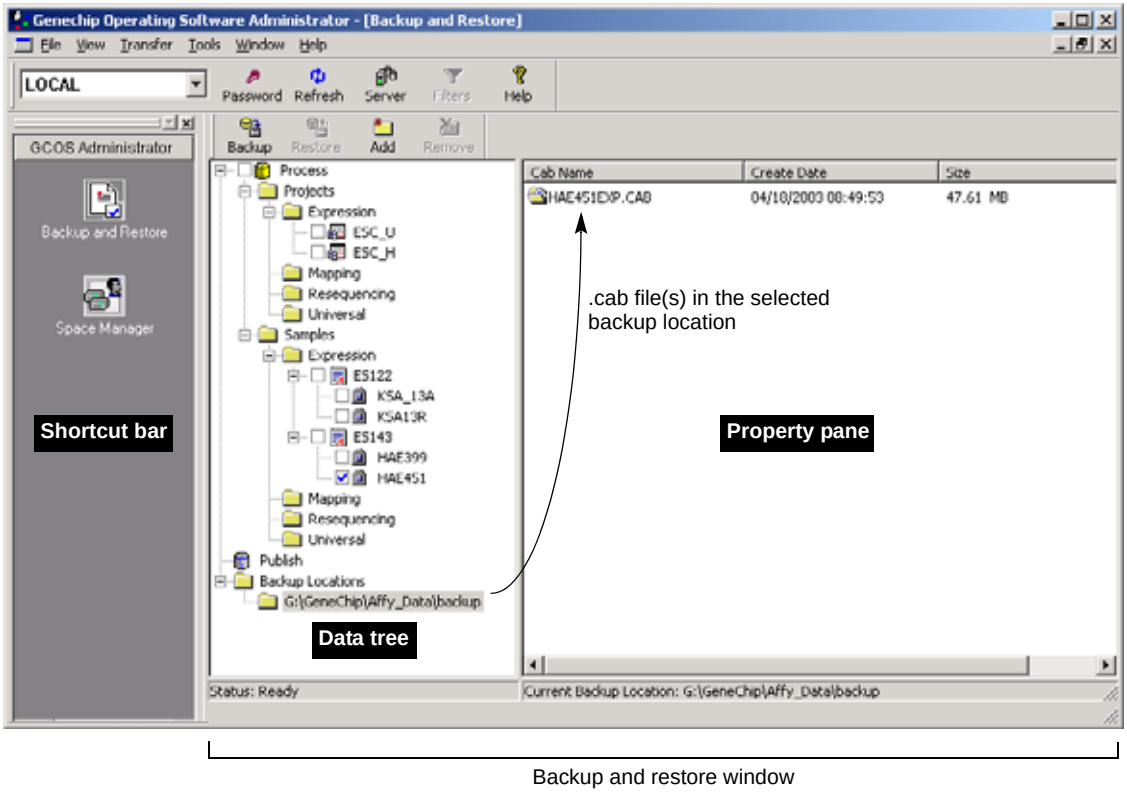


Figure 16.13
GCOS Administrator, backup and restore window

Managing Backup Locations

Cab files are saved to a user specified backup location.You can add or remove backup locations from the Backup Locations directory in the data tree.

Adding a Backup Location

1. Click the **Add** button .

   Alternatively, right-click the Backup Locations directory in the data tree and select **Add Backup Location** from the shortcut menu, or select **Transfer →Add Backup Location** from the menu bar.

   ⇒ The Browse for folder box appears **(Figure 16.14)**.

Figure 16.14
Browse for Folder box

2. Navigate to the folder of interest.
3. Click **OK**.

   ⇒ The data tree displays the new backup location.

Removing a Backup Location

To remove a backup location from the data tree, do either of the following:

- In the data tree, right-click the backup location that you want to remove, and select **Remove Backup Location** from the shortcut menu.
- In the data tree, select the backup location and click the **Remove** button . Alternatively select **Transfer →Remove Backup Location** from the menu bar.

Working with CAB Files

In the backup and restore window, you can:

- view .cab file properties
- restore a .cab file to a user-specified workstation drive or GCOS server (data transfer)
- delete a .cab file

To display the backup and restore window, click the **Backup and Restore** button in the shortcut bar .

Viewing CAB File Properties

1. In the data tree, click the backup location for the .cab of interest.

   ⇒ The property pane displays the .cab files at the selected backup location **(Figure 16.15)**.

2. Right-click the .cab of interest and select **Properties** from the shortcut menu that appears.

   ⇒ The .cab file properties are displayed **(Figure 16.15)**.

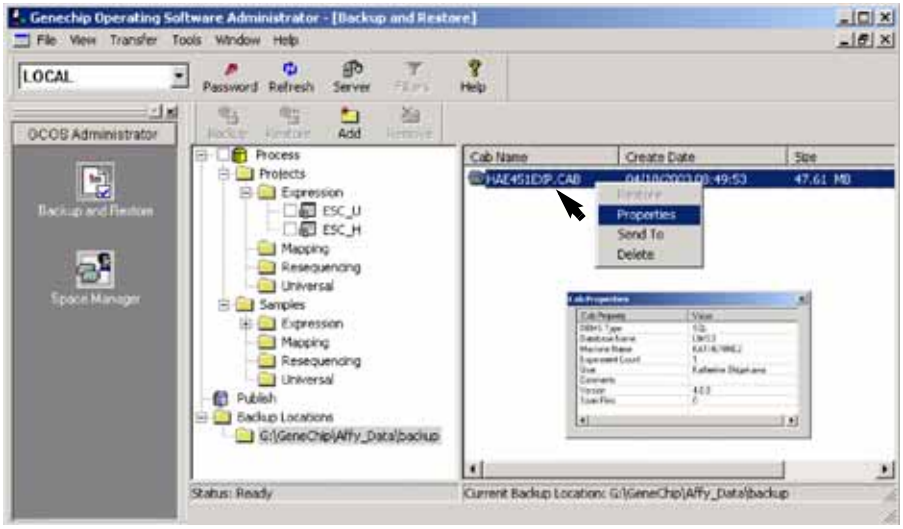


Figure 16.15
Backup and restore window, .cab file properties

Restoring a CAB File

GCOS Administrator can restore a .cab file to the process or publish database on the workstation or GCOS server. In this way, you can easily transfer a database or data to a new location.

If you are using GCOS with a GCOS server, certain privileges are required to restore a .cab file. To restore a .cab file (that you created) on a GCOS server, you must have the IMPORT PROCESS DATA privilege on the GCOS server. To restore a .cab file created by another user on the GCOS server, you must have the IMPORT PROCESS DATA privilege with other user data access rights set on the GCOS server. These privileges are not required to restore a .cab file on the GCOS workstation.

✔ Note

*It is recommended that users **not publish and restore** to the same publish database simultaneously. Sometimes the restore will be rejected and sometimes the publishing will be rejected. If the restore of a publish database fails, an error message is displayed.*

1. In the data tree, navigate to the Backup Locations directory and click the backup location of the .cab file that you want to restore.

   ⇒ The property pane displays the .cab files at the selected backup location.

2. In the property pane, click the .cab that you want to restore and click the **Restore** button . Alternatively, select **Tools** →**Restore** from the menu bar.

   ⇒ The Restore Job Information dialog box appears and displays the backup location and name for the selected .cab **(Figure 16.16)**.

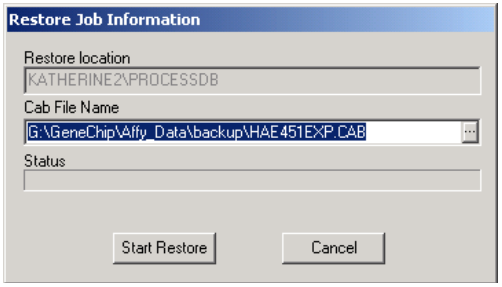


Figure 16.16
Restore Job Information dialog box

Note

*You can omit step1 and 2, and click the **Restore** button. The Restore Job Information dialog box appears, with the Cab File Name field set to the current backup location.*

3. If there is more than one available restore location, make a selection from the drop-down list.
4. Confirm the backup location and name of the .cab file that you want to restore.
5. Click **Start Restore**.

   ⇒ The restoration proceeds and the status pane displays messages about the status of the task.

   Experiments and files restored to a GCOS server from a backup .cab file have the same ownership as they had on the system from which they originated. Unless you are the owner of the restored data, you will need to set access permission.

Note

You must be a member of the "GCOS Administrator role" in order to assign/assume ownership of objects owned by role members that are not in your role(s), or objects currently owned by users that are not defined in the GCOS system because the data was restored from a .cab file backup from another system.

To set access permission, use the GCOS Manager tool to update the Role information for the data owner to allow proper access (see *Defining Privileges*, on page 361 for more information). Alternatively, use GCOS Manager to take or assign data ownership (see *Changing Data Ownership*, on page 288 for more information).

Note

After a database or data object is restored, the .cab remains in the backup location.

Copying a CAB File

You can copy a .cab file to another workstation drive or a GCOS server using the Send To feature. The .cab file is copied to the user-specified location, but the data or database is not restored.

If you are using GCOS connected to a GCOS server, certain privileges are required to copy .cab files. To copy a .cab file (that you created) to a GCOS server, you must have the IMPORT PROCESS DATA privilege on the GCOS server. To copy a .cab file created by another user to a GCOS server, you must have the IMPORT PROCESS DATA privilege with other user data access rights on the GCOS server.

To copy a .cab file (that you created) from a GCOS server, you must have the EXPORT PROCESS DATA privilege set on the GCOS server. To copy a .cab file created by another user from a GCOS server, you must have the EXPORT PROCESS DATA privilege with other user data access rights set on the GCOS server.

✔ Note *A .cab file cannot be copied using Microsoft® Windows Explorer.*

1. In the data tree, click the backup location of the .cab that you want to copy.
   ⇒ The property pane displays the .cab files at the selected backup location.

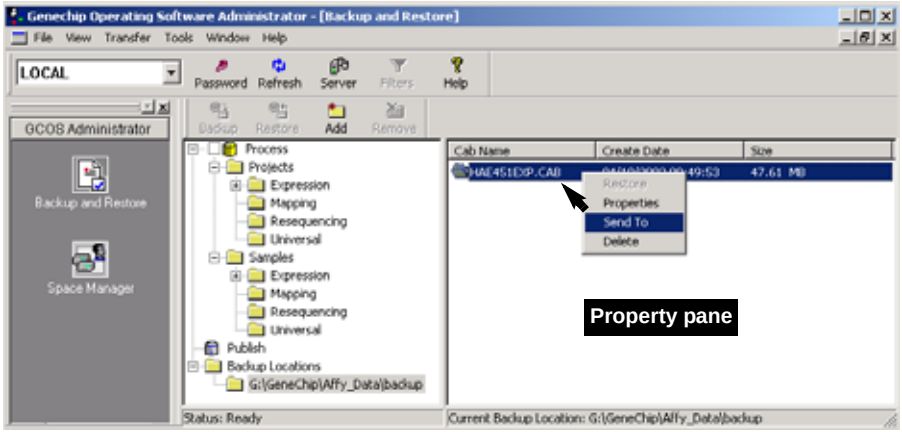


Figure 16.17
Backup and restore window, send a .cab file

2. Right-click the .cab of interest and select **Send To** from the shortcut menu.

   ⇒ The Browse for Folder box appears **(Figure 16.18)**.

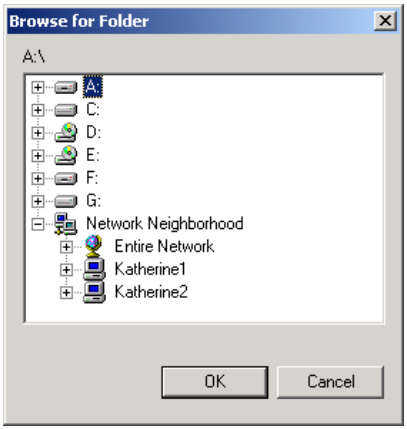


Figure 16.18
Browse for Folder box

3. Select the folder where you want to send a copy of the .cab file and click **OK**.

Deleting a CAB File

You can delete a .cab file from a workstation drive or a GCOS server. If you are using GCOS connected to a GCOS server, certain privileges are required to delete .cab files. To delete a process database .cab file (that you created) from a GCOS server, you must have the DELETE PROCESS DATA privilege on the GCOS server. To delete a process database .cab file created by another user from a GCOS server, you must have the DELETE PROCESS DATA privilege with other user data access rights on the GCOS server.

If you want to delete a publish database .cab file that you created from a GCOS server, you must have the DELETE PUBLISH DATA privilege on the GCOS server. To delete a publish database .cab file created by another user from a GCOS server, you must have the DELETE PUBLISH DATA privilege with other user data access rights on the GCOS server.

1. In the data tree, click the backup location of the .cab file that you want to delete.

   ⇒ The property pane displays the .cab files at the selected backup location.

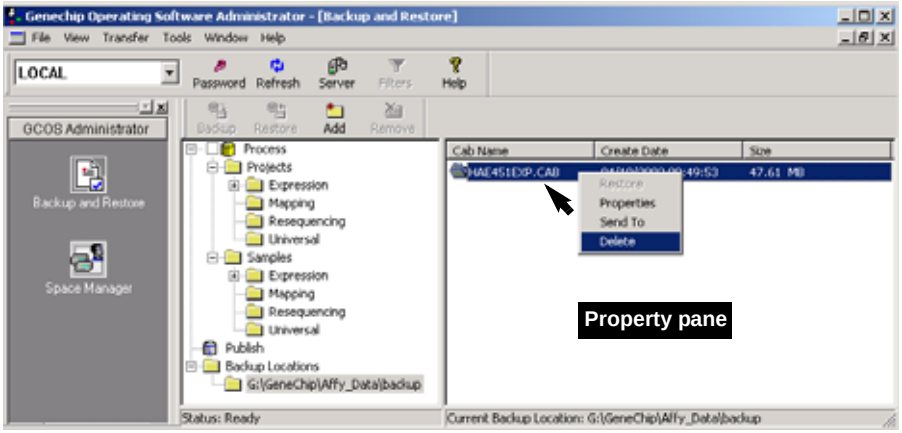


Figure 16.19
Backup and restore window, delete a .cab file

2. Right-click the .cab of interest and select **Delete** from the shortcut menu that appears **(Figure 16.19)**.

   ⇒ A warning/confirmation box appears.

3. Click **Yes** to delete the selected .cab.

Managing Workstation Databases

GCOS Administrator provides tools to help you manage your process and publish databases on the workstation.

- To open the space management window **(Figure 16.20)**, click the **Space Manager** button .

In this window, you can:

- view the properties of a workstation database, drive, or GeneChip directory
- monitor free and used space on a workstation database, drive, or directory
- relocate a database between workstation drives

Note *The Space Manager functions are only available for the workstation.*

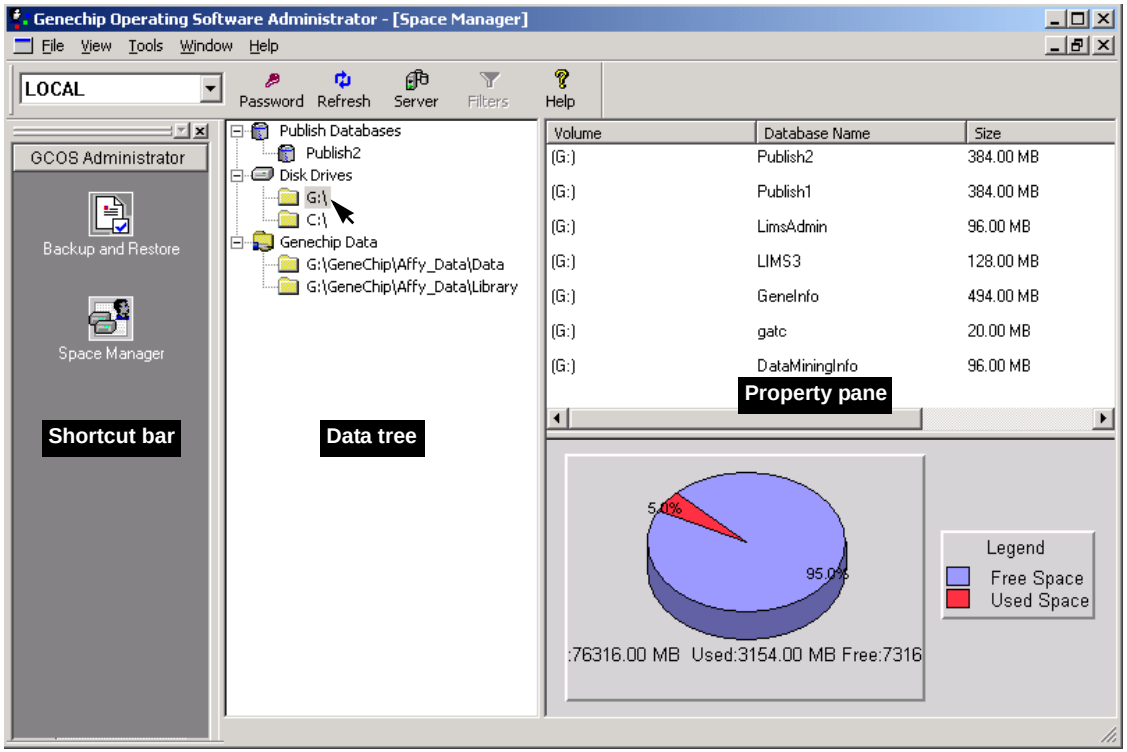


Figure 16.20
GCOS Administrator, Space Manager window

Viewing Property & Space Information

- To view information about a workstation database, drive, or directory, click the item of interest in the data tree.

⇒ The property pane displays information about the selected item (top) and a pie chart of space information.

Table 16.3 lists the types of information that are available.

Table 16.3
Space management information

Item	Property Pane Displays...
Database	Database files names Physical location of the database files Disk space allocated to each database file Pie chart of free and used database space
Drive	Databases on the drive Size of each database Create date for each database Pie chart of free and used drive space
GeneChip Data Directory	Number of files in the directory Directory size Pie chart of drive size and data size

Relocating a Publish Database

GCOS Administrator can move a workstation publish database to a user-specified drive.

1. In the data tree, click the publish database that you want to relocate.

   ⇒ The property pane displays the location and space allocation for the files that comprise the database (data device, log device, and index device) **(Figure 16.21)**.

   For more information on these files, see *Performance Tuning for SQL Server Databases*, on page 629.

2. In the property pane, make a selection from the Location drop-down list for the database file that you want to move.

3. Click outside the drop-down list (to lose focus).

   ⇒ The file is relocated to the selected drive.

4. Repeat step 2 and step 3 for each database file to be moved.

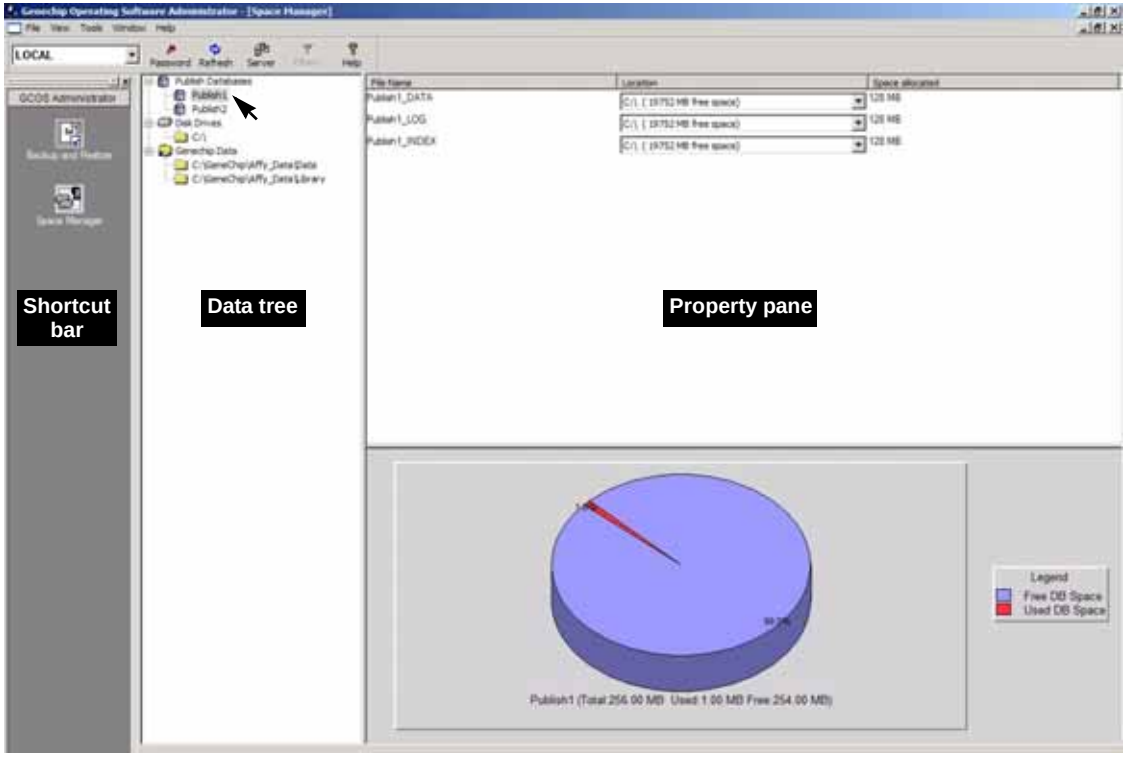


Figure 16.21
GCOS Administrator, Space Manager window

Publish Database Statistics

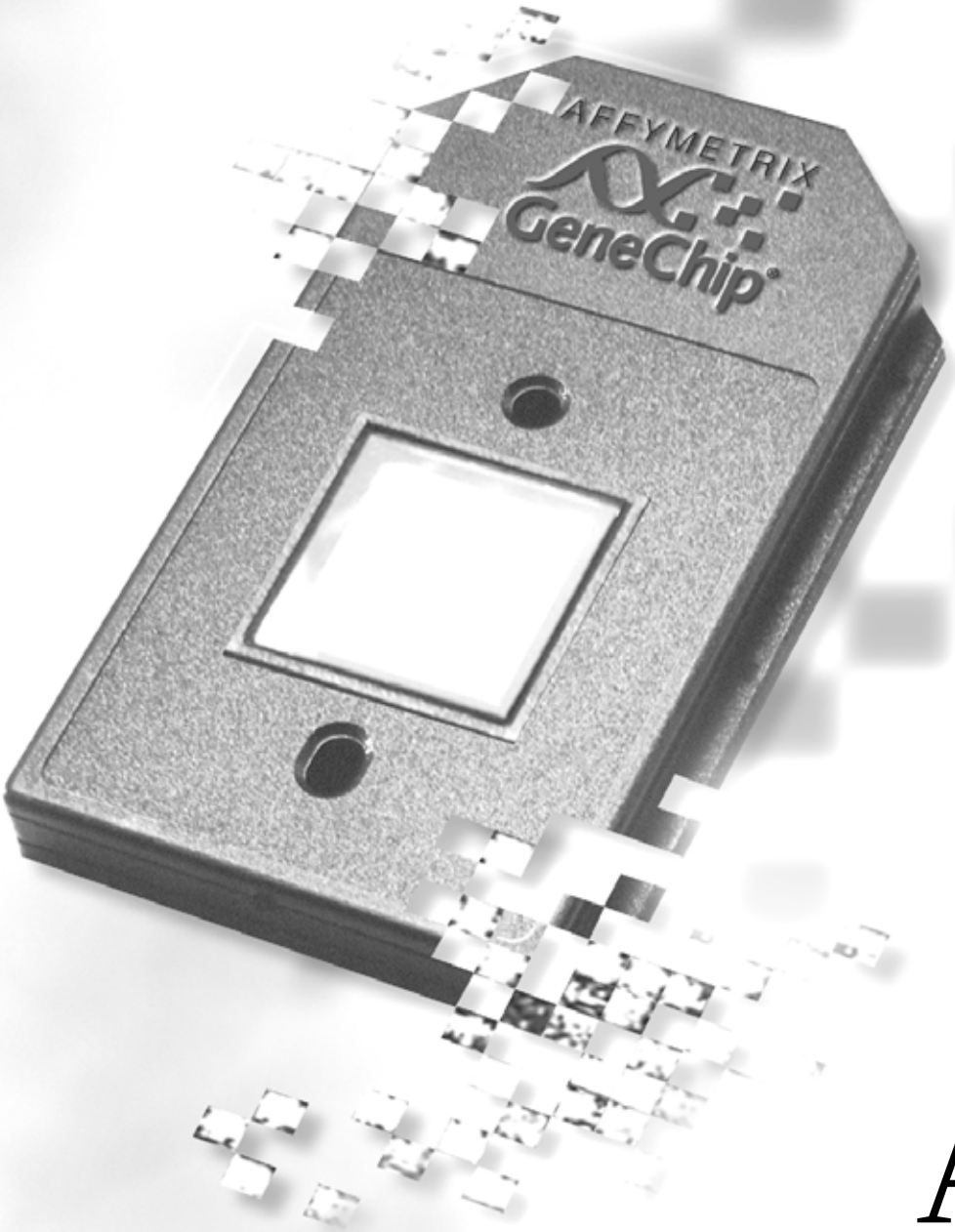
1. To view publish database statistics, click a publish database in the data tree.

   ⇒ The property pane displays all of the databases that comprise the publish database. For each database, it shows the:

   - database name
   - allocated size
   - create date
   - probe array type
   - number of published experiments

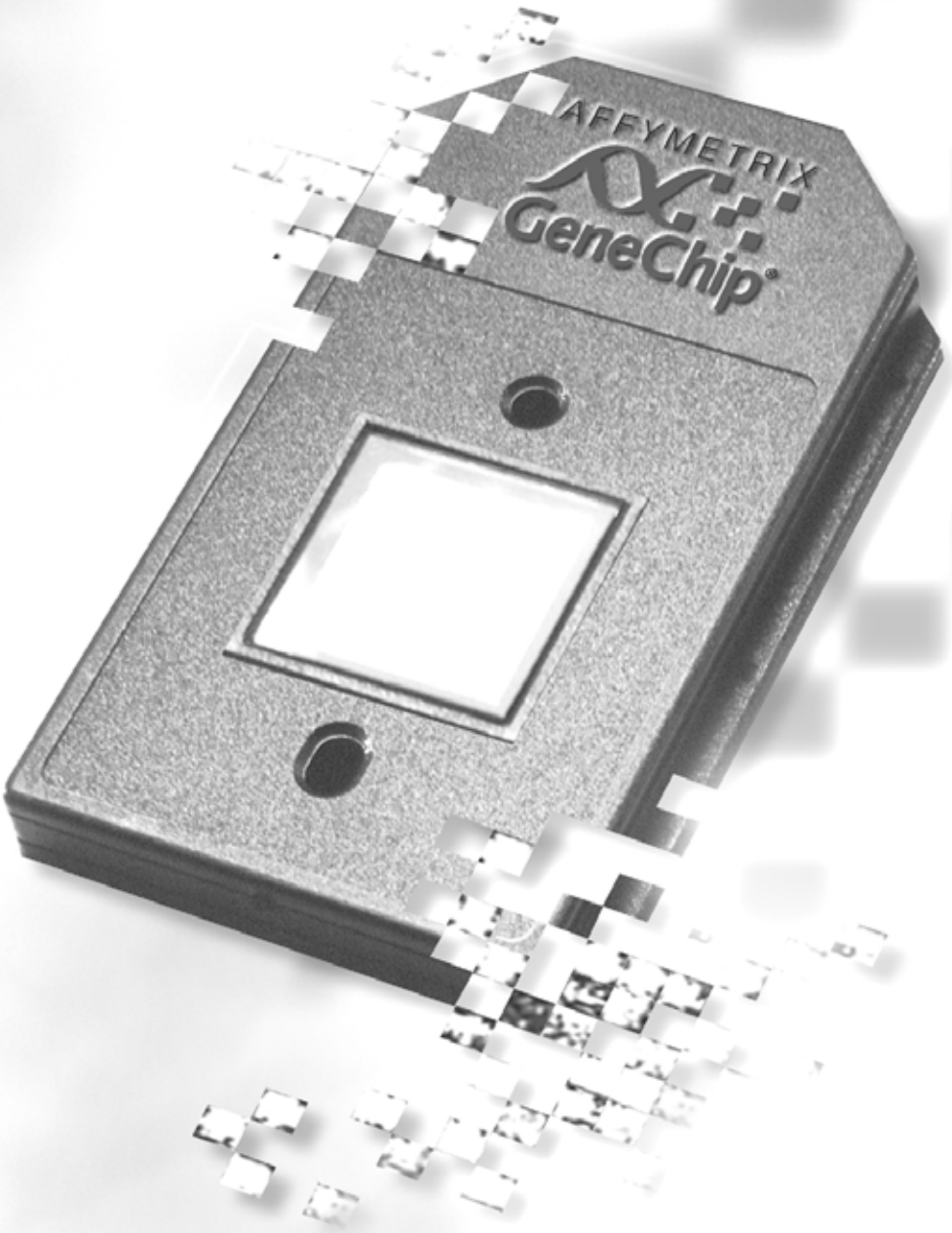
- estimated number of experiments that can be published (with intensity data)
- estimated number of experiments that can be published (without intensity data)

2. To find out how many experiments can be published using a particular probe array type, select the array type from the probe array drop-down list.



A

Appendix A



Client Testing

After installing the different client software applications, testing of the software must be completed to verify the functionality of the software components. Following the checklist below will complete the testing process.

Note

The screen captures depicted in this section may not exactly match the windows displayed on your screen.

If you encounter problems during testing, please refer to Appendix K, *Troubleshooting*, on page 635.

GCOS Manager 1.0 Testing

For more details about the GCOS Manager 1.0 client application, refer to Chapter 15 on page 275.

1. To perform the testing, login to the workstation as a user who has Administrator permissions.
2. Launch GCOS Manager 1.0 (**Start** →**Programs** →**Affymetrix** → **GCOS Manager**)
3. Verify that the a server is registered on the left hand side.
4. Click the Process, Publish, Import, Userset, Template and Roles tabs to verify that they are accessible.

Note

The Roles tab is only available when GCOS Manager is connected to a GCOS server.

Process Tab

5. Click the Process tab

   The Process tab shows the administrator the data is in the system. In this tab you can archive, unarchive, delete, export, assume ownership, and print data.

6. Expand the Samples folder.

7. Verify that the Expression folder appears.

8. Expand the Expression folder. If this is an upgrade, existing samples should appear.

Publish Tab

9. Click the **Publish** tab

   In this tab, users with access can create new publish databases or delete published data in any publish database on the system.

10. In the Publish tab, highlight the name of the active server.

11. Go to the menu bar and select **Publish →Create SQL Server Database** (if using SQL Server). If using Oracle, go to step 16

✔ Note

You can create a publish database on the workstation by following the steps for creating a database on a SQL server.

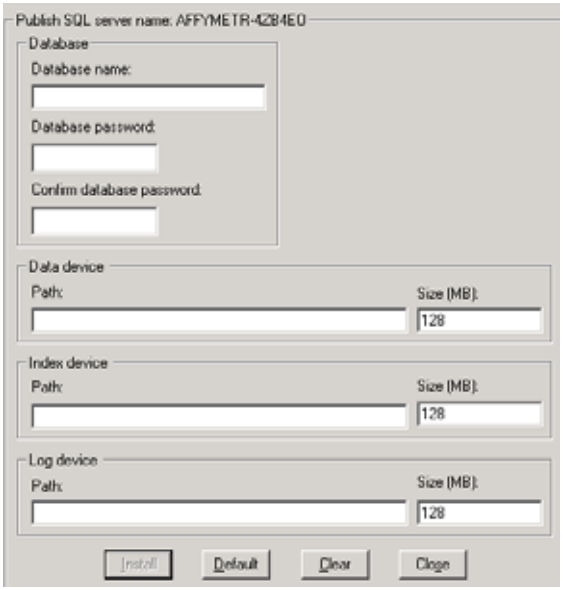


Figure A.1
Create SQL Server Publish Database window

12. Enter the following information:
    Database
    - **Database Name** - The name of the publish database to be created (in this example, MyPub).
    - **Database Password** - Enter password for access to the publish database.
    - **Confirm Database Password** - Re-enter password for confirmation.

✔ Note

The publish database is password protected. To access a publish database, users are required to enter the appropriate password.

Data Device

- **Path** – Full path location for the Data Device to reside on the GCOS server (for example, F:\GeneChipDBDevices).
- **Size** – Size of the Data Device (128MB).

Index Device

- **Path** – Full path location for the Index Device to reside on the GCOS server (i.e. F:\GeneChipDBDevices).
- **Size** – Size of the Index Device (128MB).

Log Device

- **Path** – Full path location for the Log Device to reside on the GCOS server (for example, F:\GeneChipDBDevices).
- **Size** – Size of the Log Device (128MB).

✔ Note

The Microsoft® SQL Server database that you create must be between 128MB to 1024 MB. If you want to expand the size after the database is created, use SQL Enterprise Manager to expand the database.

13. Click **Install** after all information is entered.
    This may take a few minutes depending on the size of the database created.

✔ Note

Creating a Publish database only needs to be tested once from a client. It does not need to be tested on additional clients, as publish databases are quite large. The path location for the devices must already exist on the server, otherwise creating the database will fail.

14. Click **Close** when the database is created.
15. Go to step 19.
16. To create an Oracle publish database, select **Publish →Create Oracle Database** from the menu bar.
    ⇒ Create Oracle Publish Database window **(Figure A.2)**.

✔ Note

You can create an Oracle publish database when the GCOS Manager is connected to a GCOS server running Oracle.

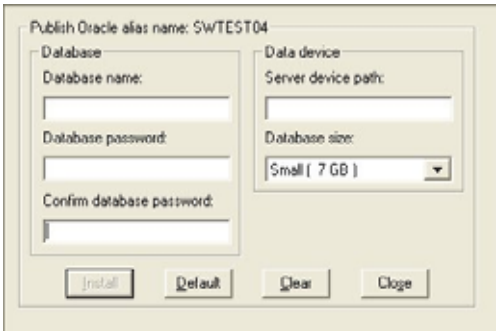


Figure A.2
Create Oracle Publish Database window

17. Enter the following information:

Database:

- **Database name** – Name of the Publish database (in this example, MyPub)
- **Database password** – Enter password for access to publish database
- **Confirm database password** – Re-enter password for confirmation

✔ Note

The publish database is password protected. To access a publish database, users will be required to enter the appropriate password.

Data Device:

- **Server device path** – Full path location for the Device to reside on the GCOS server (for example, D:\GeneChipDBDevices\)
- **Database size** – Small or Large (in this example, select **Small**)

✔ Note

The server device path directory specifies the file location on the actual publish database server. If the publish database is being created on a remote server, make sure the path specified is available on that server.

Note

*An Oracle publish database is much larger than the default SQL Server database. Verify that there is adequate space on the server before creating an Oracle database. The **Small** Oracle publish database requires 8GB of hard disk space. The **Large** Oracle publish database requires 17 GB of hard disk space. These database sizes correspond to publishing approximately 500 and 1000 experiments respectively. Creating an Oracle database will take a while.*

18. Click **Close** after the database is created.

Note

You only need to test creating a publish database once from a client. You need not repeat the test on additional clients, as a publish database is quite large.

Import Tab

19. Click the **Import** tab.

    The Import function copies or imports experiment data created in GCOS and moves the data to a process database on the workstation or GCOS server.

20. On the menu bar, select **Tools →File Path**. Set the path for the location of the files for import. For example, C:\GeneChip\TempData **(Figure A.3)**.

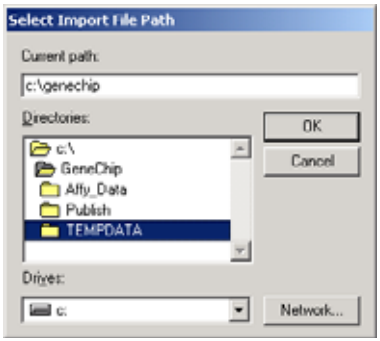


Figure A.3
Select Import File Path window

21. Click **OK**.
22. Highlight the **Expression Folder**. From the menu bar, select **Import →Create Sample**.
    ⇒ The Create Expression Sample window appears **(Figure A.4)**.

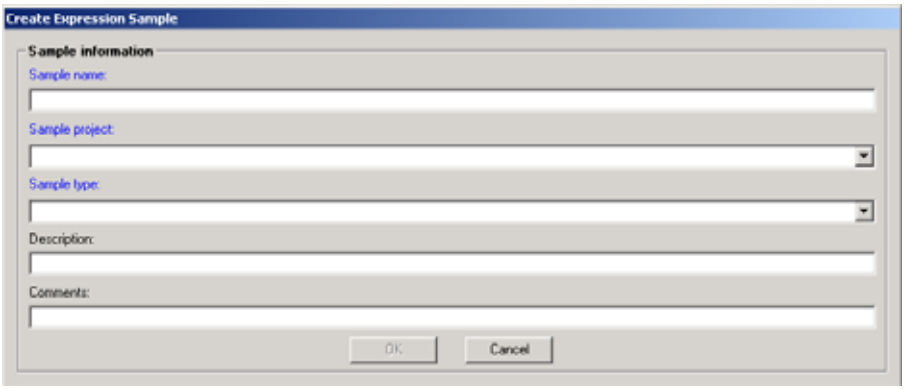


Figure A.4
Create Expression Sample window

23. Enter the following information:
    - **Sample Name**: ImportSample
    - **Sample Project**: MySampleProject
    - **Sample Type**: MySampleType
    - **Description**: <optional>
    - **Comments**: <optional>

    The fields in blue are required fields.
24. Click **OK**.

✔ Note

GCOS Manager can import any data for which a library file has been installed. To preserve the experiment information, all data in the sample name, project, and array type should match the data in the experiment file.

25. Select the .exp file and drag it to the sample in the **Data to import** pane **(Figure A.5)**.

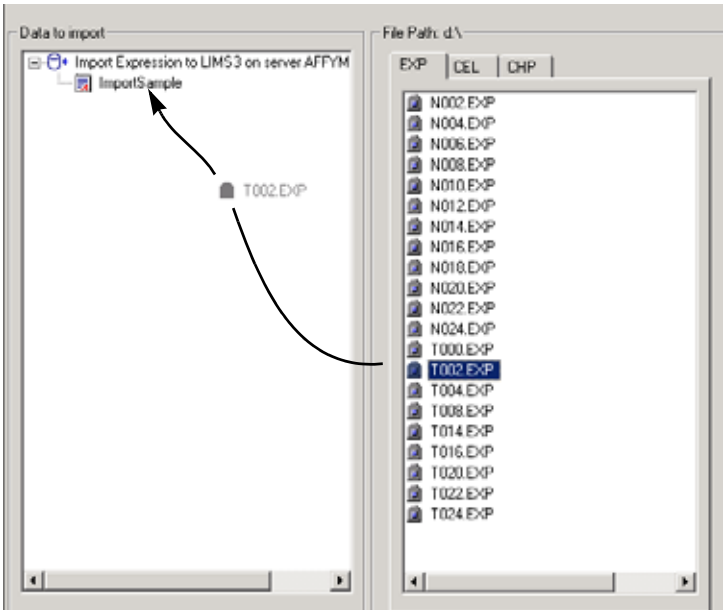


Figure A.5
Importing .exp data

⇒ The .exp file brings all associated files with the same prefix when placed on the sample **(Figure A.6)**.

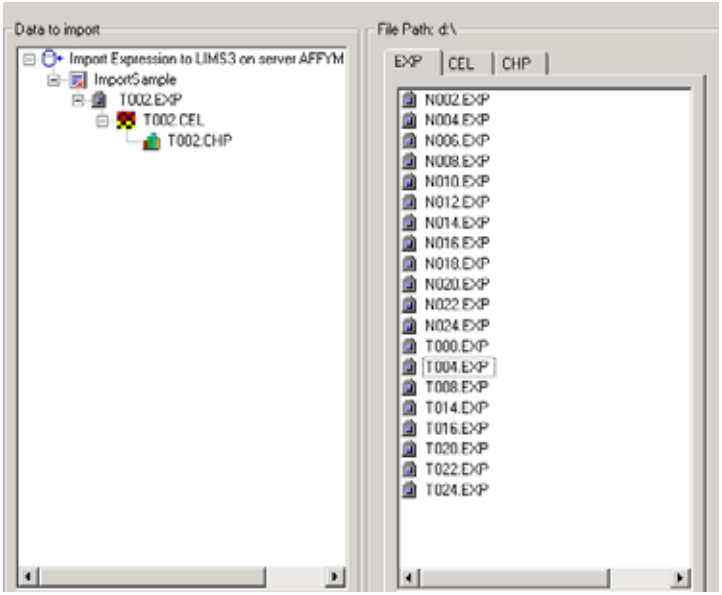


Figure A.6
Importing .exp brings associated files

26. If there are additional .cel or .chp files associated with this experiment, select the **CEL** or **CHP** tab and drag the appropriate file to the sample in the **Data to import** pane.
27. When all of the files for the import are associated with a sample, select **Import →Start Import** from the menu bar. Alternatively, right-click any file in the **Data To Import** pane and select **Start Import** from the shortcut menu that appears.
28. If the import fails, check the import status in status pane. Double-click **Import** in Task View and review the error message.

Userset Tab

29. Click the **Userset** tab.

    In this tab you can create a userset that defines values for the user-modifiable expression algorithm parameters. You can select the userset by name to analyze experiments using the same set of algorithm parameter settings.

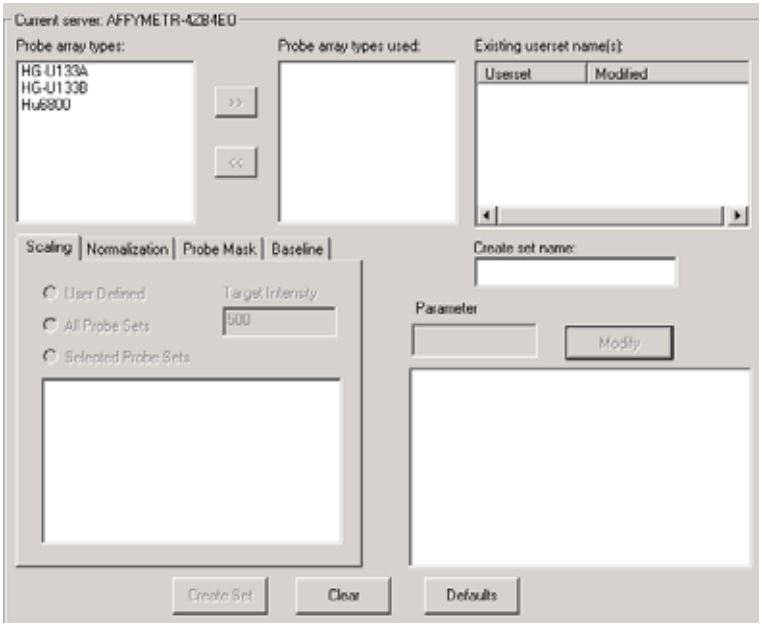


Figure A.7
Upper right pane of the Userset tab

30. Enter a name for the set in the **Create Set Name** field (enter **MySet**).
31. Select the Probe Array Type (**Hu6800**) in the **Probe Array Types Used** field and move it by pressing >>. (Only one probe array type can be moved at a time.)
32. Click **Create Set**. The userset is created and the userset name is added to the right hand side of the pane.

Template Tab

✔ Note

*Template attributes cannot be deleted, only deactivated. A template can be deleted (right-click the template and select **Delete** from the shortcut menu that appears).*

33. Click the **Template** tab.

    In this tab the administrator can add fields to the **Experiment Template** and the **Sample Template** that are used in GCOS.

34. To create an **Experiment Template**, highlight the Experiment Templates Folder.

35. Select **Template →Create** from the menu bar. (If it is grayed out, highlight the Experiment Templates Folder again, or right-click the Experiment Template Folder and select **Create** from the shortcut menu that appears) **(Figure A.8)**.

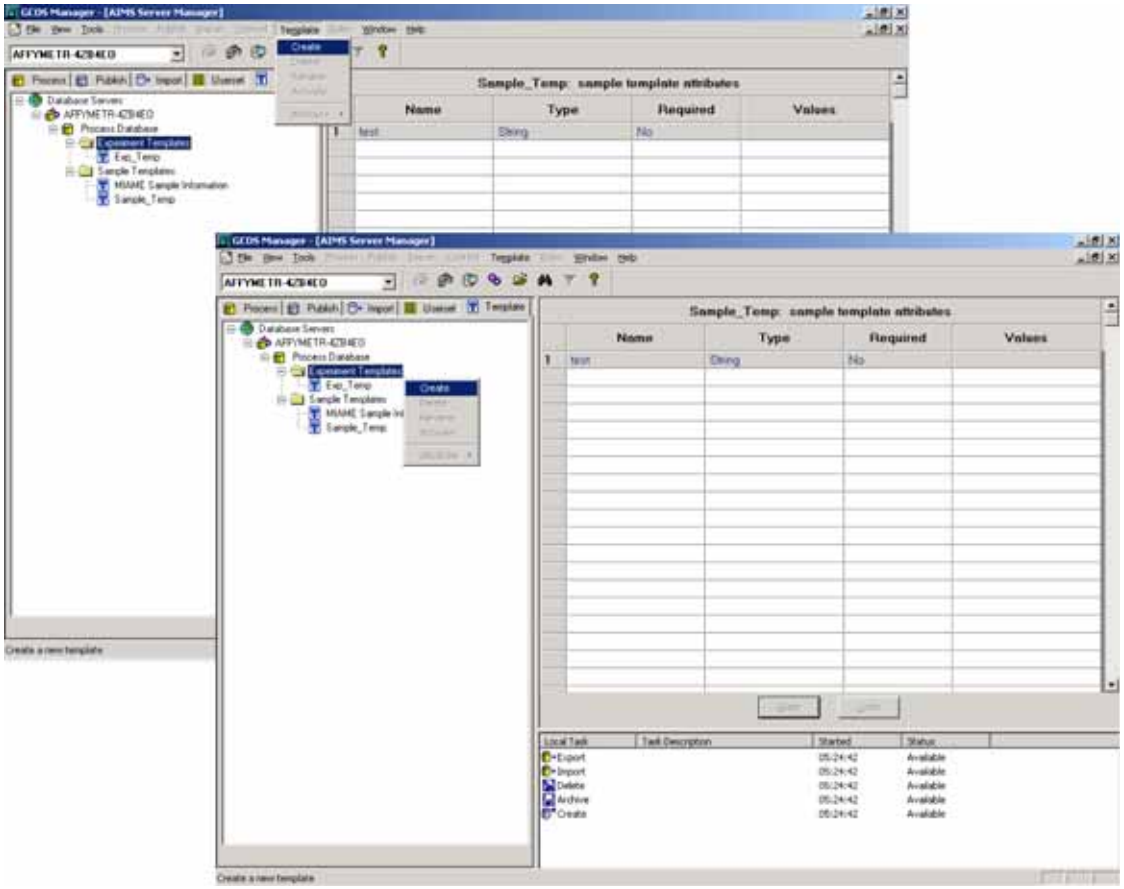


Figure A.8
Template Tab window

⇒ The Create Template box appears **(Figure A.9)**.

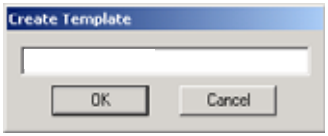


Figure A.9
Create Template box

36. Enter the name of the template (for example, exp_temp). Click **OK**.

    ⇒ The experiment template attributes window appears in the upper right pane **(Figure A.10)**.

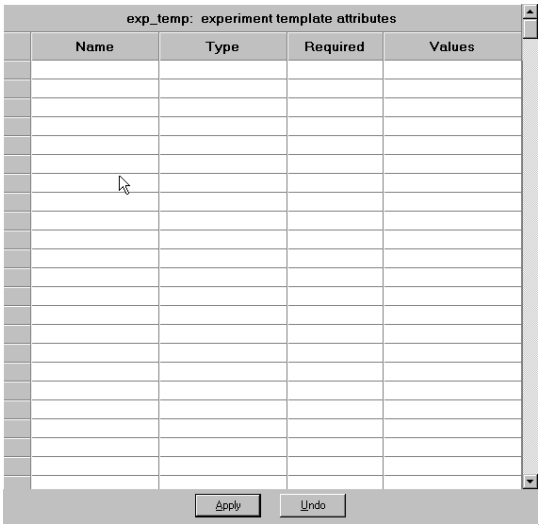


Figure A.10
Experiment Template Attributes window

37. Double-click in the **Name** field to get a blinking cursor.
38. Enter *Comments* in the **Name** field.
39. Select **String** in the **Type** field.
40. Select **No** in the **Required** field.

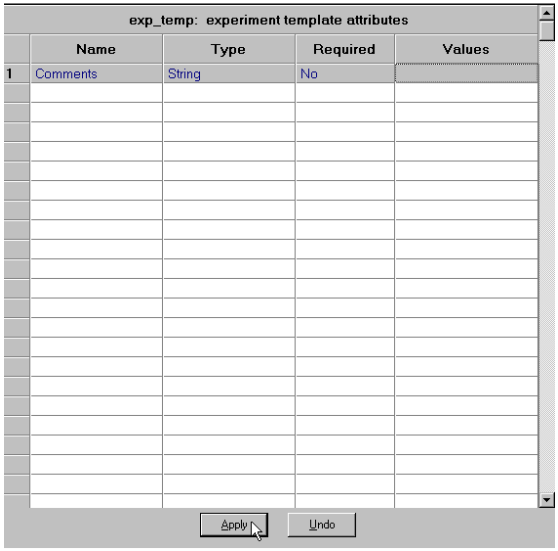


Figure A.11
Attributes and values entered

41. Click **Apply**.

    ⇒ The Exp_Temp experiment template is added to the Experiment Templates list.
    (You must select the Experiment Templates folder to see the newly added template.)

42. To create a **Sample Template**, highlight the Sample Templates Folder.

43. Select **Template →Create** from the menu bar. (If it is grayed out, highlight the Sample Templates folder again, or right-click the Sample Template folder and select **Create** from the shortcut menu that appears.) **(Figure A.12)**

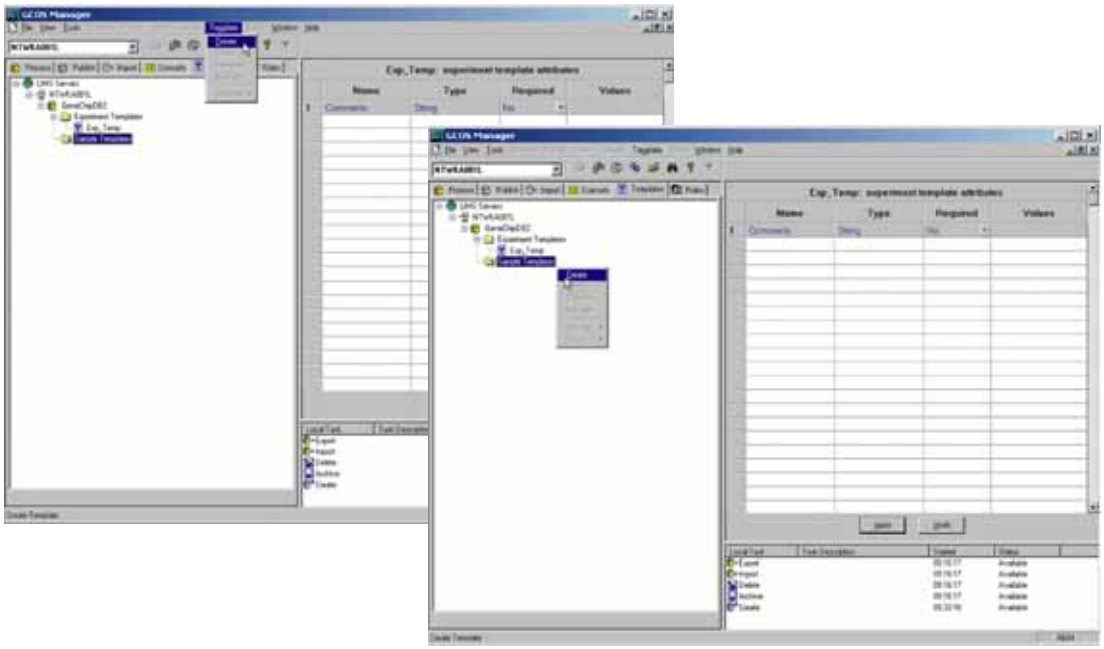


Figure A.12
Sample Template window

⇒ The Create Template window appears **(Figure A.13)**.

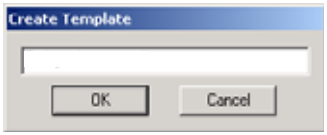


Figure A.13
Create Template window

44. Enter the name of the template (for example, *Sample_Temp*). Click **OK**.

    ⇒ The sample template attributes window appears **(Figure A.14)**.

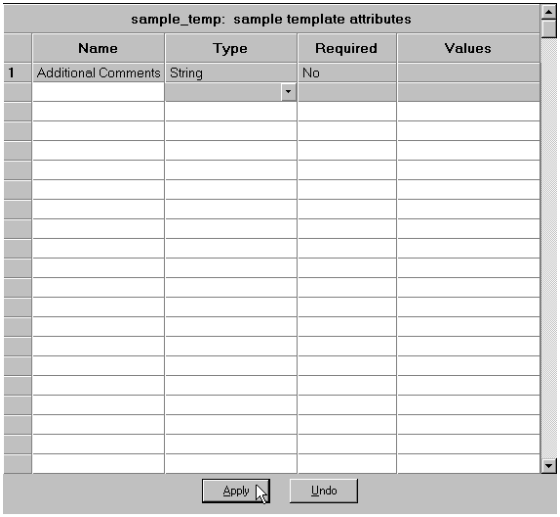


Figure A.14
Sample Template Attributes window

45. Double-click in the **Name** field to get a blinking cursor.
46. Enter *Additional Comments* in the **Name** field.
47. Select **String** in the **Type** field.
48. Select **No** in the **Required** field.

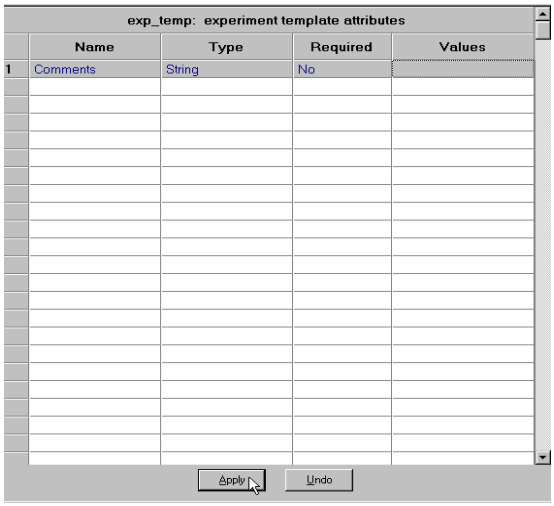


Figure A.15
Attributes and Values Entered

49. Click **Apply**.

    ⇒ The **Experiment** and **Sample** templates are created and appear in the left hand pane **(Figure A.16)**.

Note

You must select the Experiment or Sample template folder to see the list of available templates.

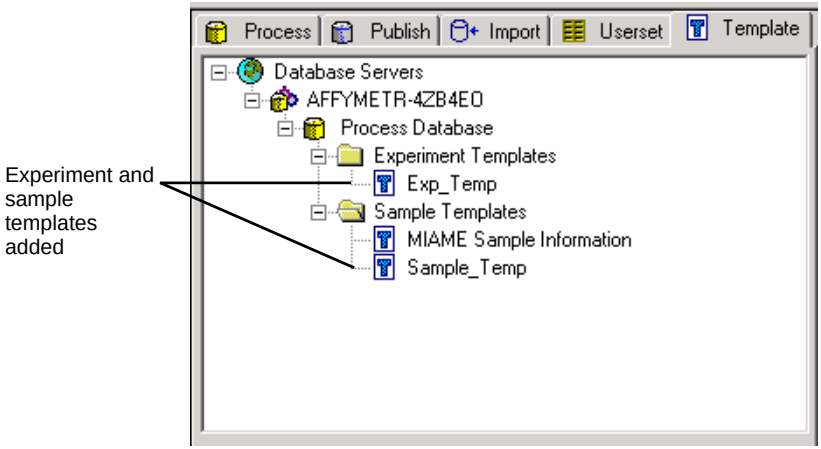


Figure A.16
Experiment and Sample Templates Added

Roles Tab

Note *The roles tab is only available when using GCOS Manager with a registered GCOS server.*

50. Click the **Roles** tab.

    The **Roles** tab is used to set up security for the end-users. Therefore, the functions of the Roles tab are solely for the use of the system administrator and the tab only appears for users who have been granted the **Maintain Roles** privilege.

51. Click **GCOS Server Administrator** from the left hand pane under the name of the GCOS server.

    ⇒ The function of the GCOS Server Administrator is displayed. All privileges are granted for the administrator **(Figure A.17)**.

Function	Other User Data Access
☑ READ PROCESS DATABASE	Yes
☑ UPDATE PROCESS DATABASE	Yes
☑ UPDATE PUBLISH DATABASE	Yes
☑ ARCHIVE PROCESS DATA	Yes
☑ DELETE PROCESS DATA	Yes
☑ DELETE PUBLISH DATA	Yes
☑ IMPORT PROCESS DATA	Yes
☑ EXPORT PROCESS DATA	Yes
☑ TAKE OWNERSHIP PROCESS DATA	Yes
☑ MAINTAIN ROLES	Yes
☑ DELETE SERVER TASKS	Yes
☑ MAINTAIN TEMPLATES	Yes
☑ MAINTAIN USERSETS	Yes
☑ CREATE PUBLISH DATABASE	Yes

Figure A.17
Administrator Privileges window

52. Click **GCOS Server User** from the left hand pane under the GCOS server name.

    ⇒ The function of the GCOS Server User is displayed.

    By default, all privileges are granted for the User except for the last five privileges **(Figure A.18)**.

Function	Other User Data Access
☑ READ PROCESS DATABASE	Yes
☑ UPDATE PROCESS DATABASE	Yes
☑ UPDATE PUBLISH DATABASE	Yes
☑ ARCHIVE PROCESS DATA	Yes
☑ DELETE PROCESS DATA	No
☑ DELETE PUBLISH DATA	Yes
☑ IMPORT PROCESS DATA	Yes
☑ EXPORT PROCESS DATA	Yes
☐ TAKE OWNERSHIP PROCESS DATA	Yes
☐ MAINTAIN ROLES	Yes
☐ DELETE SERVER TASKS	Yes
☐ MAINTAIN TEMPLATES	Yes
☐ MAINTAIN USERSETS	Yes
☑ CREATE PUBLISH DATABASE	Yes

Figure A.18
Default User Privileges

53. Under the GCOSServer3 database, expand the **GCOS Server Administrator** group and **GCOS Server User** group.

    ⇒ The domain name\Global Group appears and displays the group members **(Figure A.19)**.

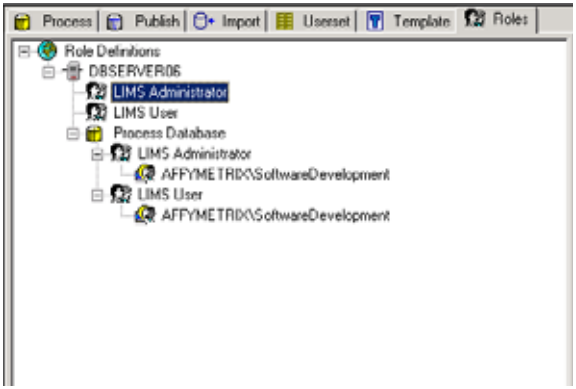


Figure A.19
Roles Tab window

54. Click the right hand pane to expand the domain and the list of Global Groups.
55. Expand the GCOS Server Global Group.
56. Drag a specific user to the **GCOS Server User** or **GCOS Server Administrator** group.

    This allows individual users or Global Groups access to certain functions on the GCOS server.
57. Click any existing role in the left pane.
58. In the Create Role dialog box, type a name for a new role.
59. Click **OK**.

    ⇒ The new role appears in the left pane under the server.

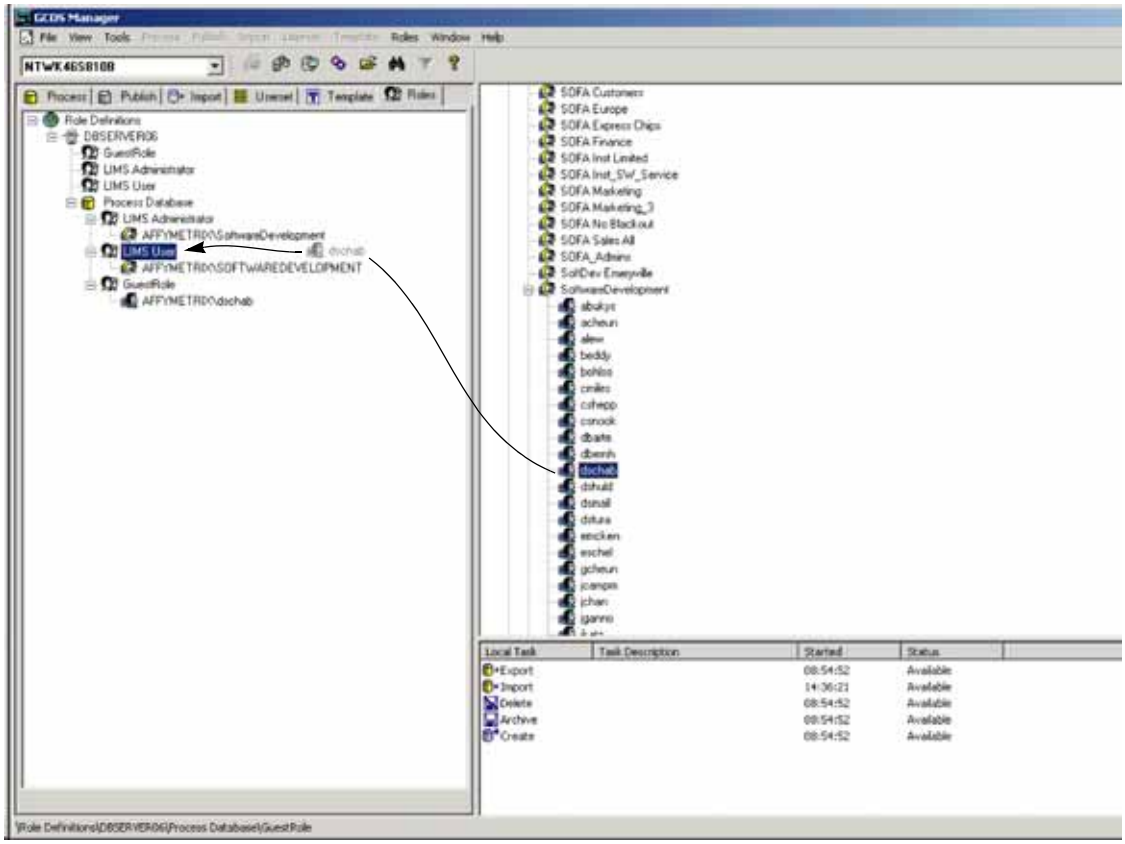


Figure A.20
Roles Tab window

Event Viewer and Server Tasks

60. To display the Event Viewer for the GCOS server, select **Tools →Server Event Viewer** from the menu bar.

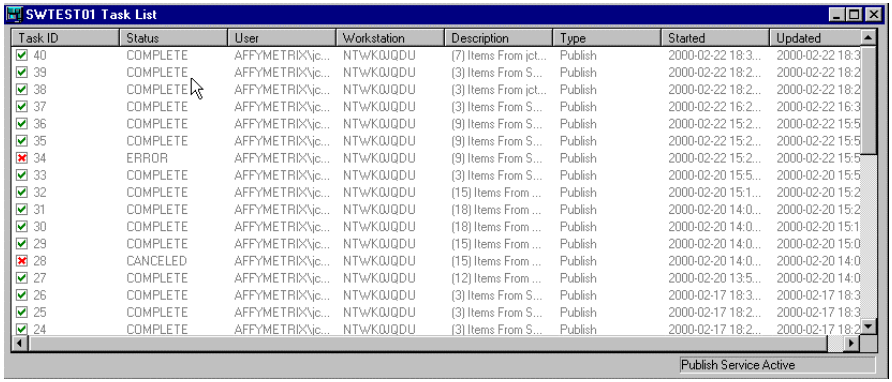
    ⇒ The **Event Viewer** for the GCOS server is displayed **(Figure A.21)**.

    You can view the System, Security and Application Log**.**

61. Close the Event Viewer.

62. To view the Server Tasks, select **Tools →Server Tasks** from the menu bar.

    This monitors and displays the status of any published data and grows as more data are published.



Task ID	Status	User	Workstation	Description	Type	Started	Updated
40	COMPLETE	AFFYMETRIX\jc...	NTWKQUQDU	(7) Items From jct...	Publish	2000-02-22 18:3...	2000-02-22 18:3
39	COMPLETE	AFFYMETRIX\jc...	NTWKQUQDU	(3) Items From S...	Publish	2000-02-22 18:2...	2000-02-22 18:2
38	COMPLETE	AFFYMETRIX\jc...	NTWKQUQDU	(3) Items From jct...	Publish	2000-02-22 18:2...	2000-02-22 18:2
37	COMPLETE	AFFYMETRIX\jc...	NTWKQUQDU	(3) Items From S...	Publish	2000-02-22 16:2...	2000-02-22 16:3
36	COMPLETE	AFFYMETRIX\jc...	NTWKQUQDU	(9) Items From S...	Publish	2000-02-22 15:2...	2000-02-22 15:5
35	COMPLETE	AFFYMETRIX\jc...	NTWKQUQDU	(9) Items From S...	Publish	2000-02-22 15:2...	2000-02-22 15:5
34	ERROR	AFFYMETRIX\jc...	NTWKQUQDU	(9) Items From S...	Publish	2000-02-22 15:2...	2000-02-22 15:5
33	COMPLETE	AFFYMETRIX\jc...	NTWKQUQDU	(3) Items From S...	Publish	2000-02-20 15:5...	2000-02-20 15:5
32	COMPLETE	AFFYMETRIX\jc...	NTWKQUQDU	(15) Items From ...	Publish	2000-02-20 15:1...	2000-02-20 15:2
31	COMPLETE	AFFYMETRIX\jc...	NTWKQUQDU	(18) Items From ...	Publish	2000-02-20 14:0...	2000-02-20 15:2
30	COMPLETE	AFFYMETRIX\jc...	NTWKQUQDU	(18) Items From ...	Publish	2000-02-20 14:0...	2000-02-20 15:1
29	COMPLETE	AFFYMETRIX\jc...	NTWKQUQDU	(15) Items From ...	Publish	2000-02-20 14:0...	2000-02-20 15:0
28	CANCELED	AFFYMETRIX\jc...	NTWKQUQDU	(15) Items From ...	Publish	2000-02-20 14:0...	2000-02-20 14:0
27	COMPLETE	AFFYMETRIX\jc...	NTWKQUQDU	(12) Items From ...	Publish	2000-02-20 13:5...	2000-02-20 14:0
26	COMPLETE	AFFYMETRIX\jc...	NTWKQUQDU	(3) Items From S...	Publish	2000-02-17 18:3...	2000-02-17 18:3
25	COMPLETE	AFFYMETRIX\jc...	NTWKQUQDU	(3) Items From S...	Publish	2000-02-17 18:2...	2000-02-17 18:3
24	COMPLETE	AFFYMETRIX\jc...	NTWKQUQDU	(3) Items From S...	Publish	2000-02-17 18:2...	2000-02-17 18:2

Figure A.21
Server Tasks window

✔ Note *The task list is populated after experiment data are published.*

GCOS 1.0 Testing

Preparing and Scanning an Experiment

63. Select **File →New Experiment** from the menu bar or click the **Experiments** button .

    ⇒ The Sample and Experiments window is displayed **(Figure A.22)**.

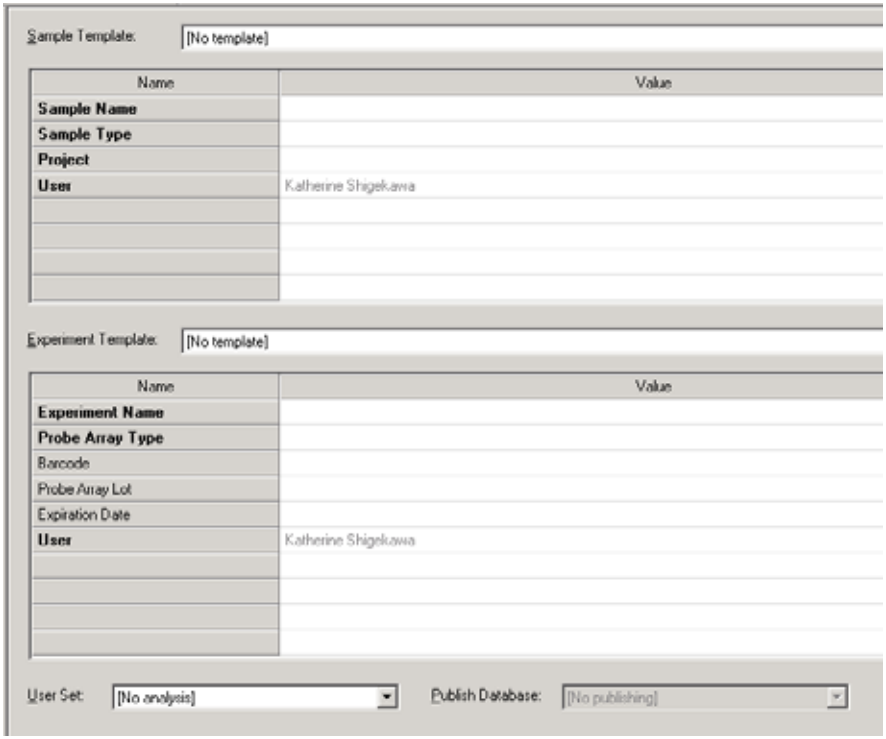


Figure A.22
Sample and Experiments window

64. Enter the following information for Experiment 1 **(Figure A.23)**:
    - **Sample Name**: *MySample*
    - **Sample Type**: *SampleType*
    - **Project**: *SampleProject*
    - **Experiment Name**: *TestExp*
    - **Probe Array Type**: *Hu6800*

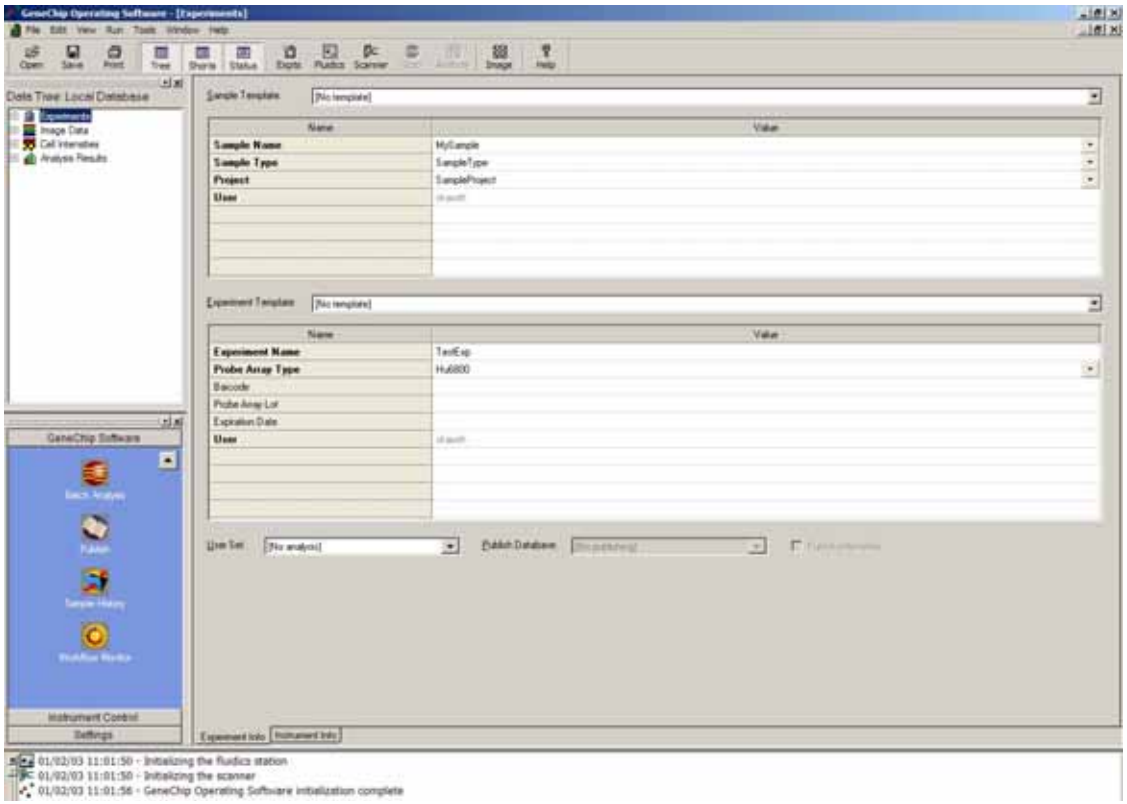


Figure A.23
Experiment 1 input

65. Save the experiment information by selecting **File →Save** from the menu bar or click the **Save** button .
66. Enter the following information for Experiment 2 **(Figure A.24)**:
    - **Sample Name**: *MySample*
    - **Sample Type**: *SampleType*
    - **Project**: *SampleProject*
    - **Experiment Name**: *TestExp_2*
    - **ProbeArray Type**: *Hu6800*
67. Select the **Analysis Set (MySet)** that was created in GCOS Manager. (Only analysis sets for the same probe array type appear.)
68. Select the **Publish Database (MyPub)** that was created in GCOS Manager.
69. Select **Publish Intensities**.

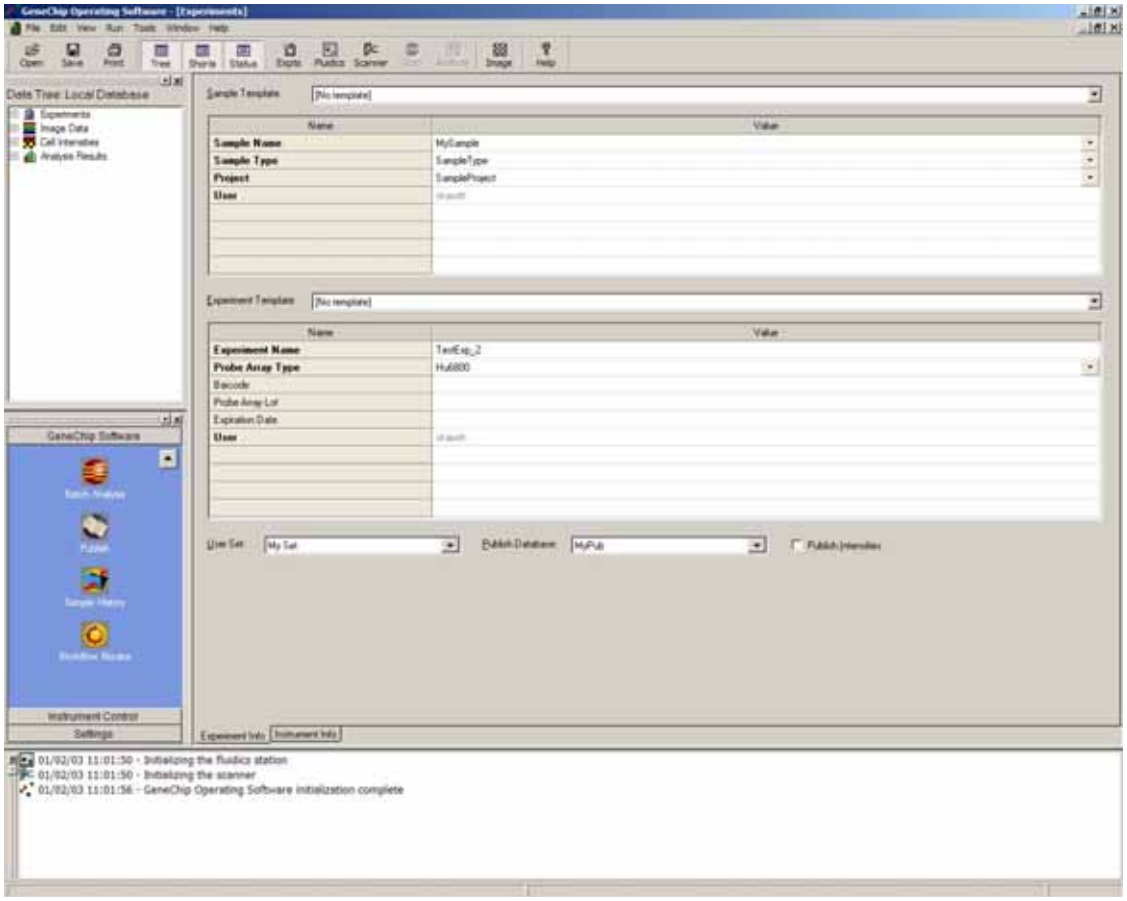


Figure A.24
Experiment 2 input

70. Save the experiment information by selecting **File** →**Save** from the menu bar or click the **Save** button.
71. A dialog box appears prompting for the publish database password. Enter the password and click **Login**.
72. From the **Sample Template** drop-down list, select **Sample_Temp**.
73. From the **Experiment Template** drop-down list, select **Exp_Temp**.

74. Enter the following information for Experiment 3 **(Figure A.25)**:
    - **Sample Name**: *MySample*
    - **Sample Type**: *SampleType*
    - **Project**: *SampleProject*
    - **More Comments**: *This is a test.*
    - **Experiment Name**: *TestExp_3*
    - **ProbeArray Type**: *Hu6800*
    - **Comments**: *Additional comments*
75. Set Analysis Set and Publish Database to [No analysis] and [No publish] respectively.
76. De-select **Publish intensities**.

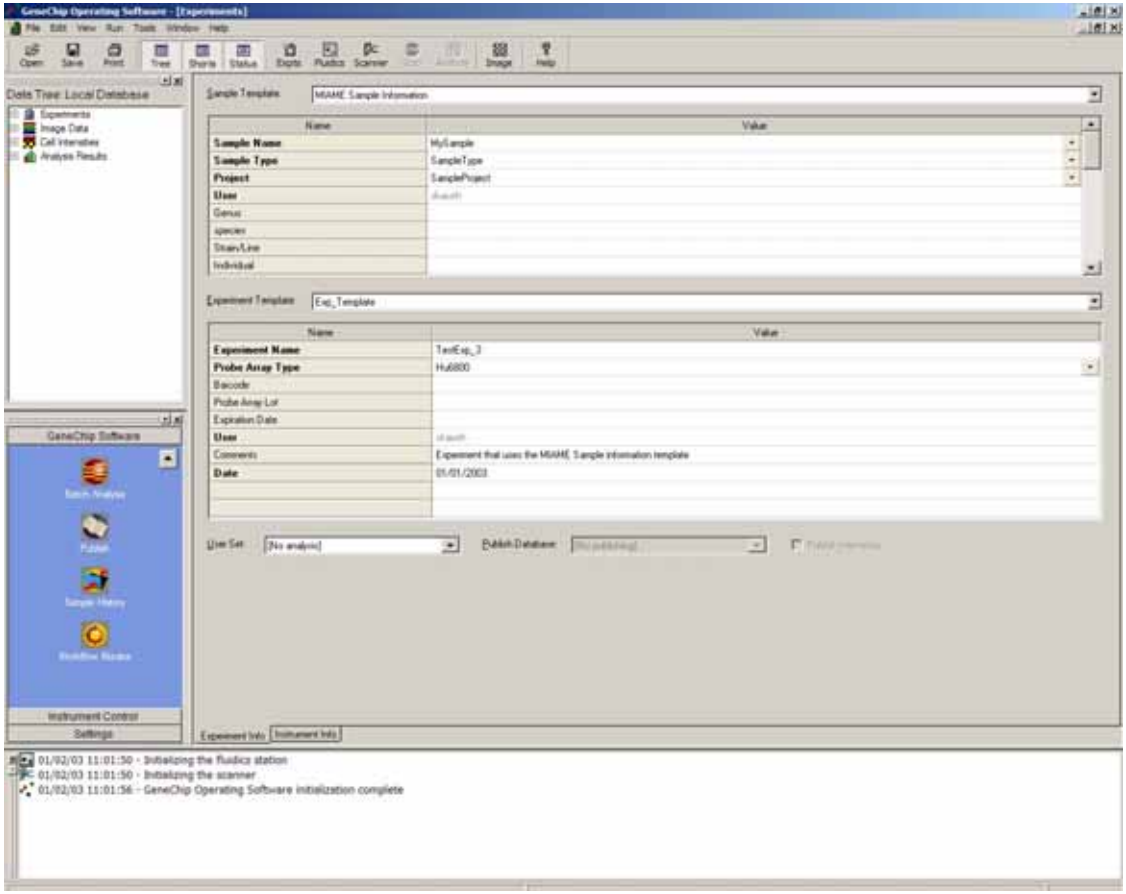


Figure A.25
Experiment 3 input

77. Save the experimental information by selecting **File →Save** from the menu bar or click the **Save** button.

Hybridizing an Experiment

78. To hybridize an experiment, select **Run →Fluidics** from the menu bar or click **Instrument Control** in the shortcut bar and click the **Fluidics** button .
79. Select **Module 1** **(Figure A.26)**.

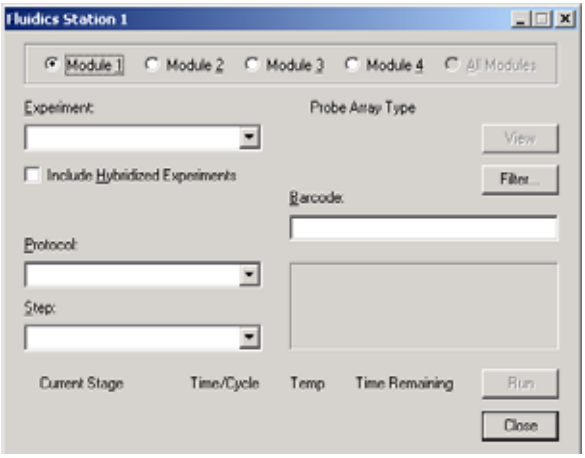


Figure A.26
Fluidics Station window

80. In the **Experiment** drop-down box, select **TestExp**.
81. In the **Protocol** drop-down box, select the protocol to run the experiment on. (Select the appropriate fluidics protocol for the probe array type.) **(Figure A.27)**.

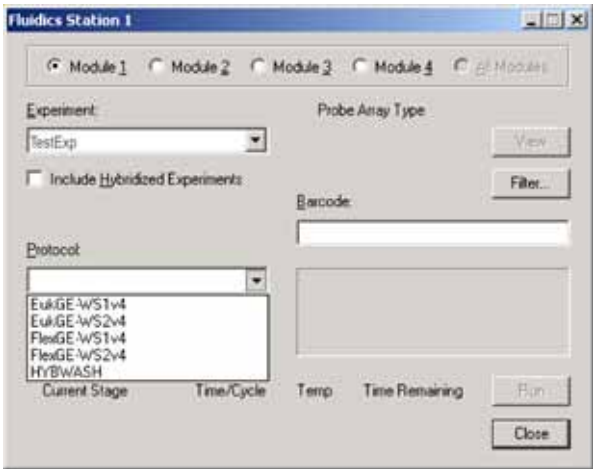


Figure A.27
Select Protocol

82. Click **Run**.

⇒ The Fluidics Station window displays the **Current Stage** of the hybridization **(Figure A.28)**. This takes a few minutes.

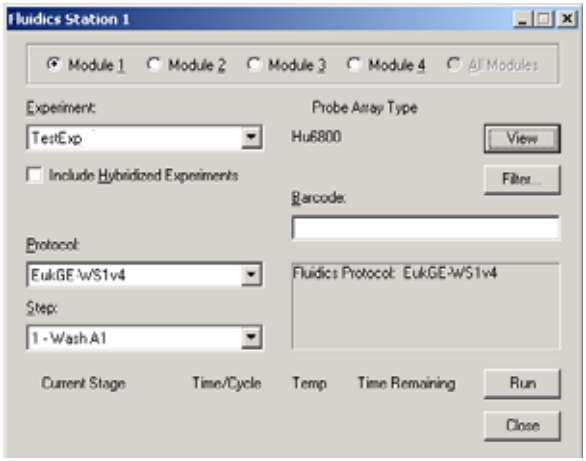


Figure A.28
Hybridizing experiment

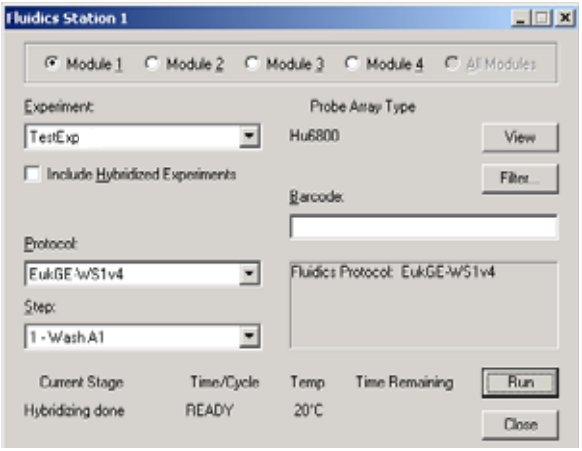


Figure A.29
Hybridization complete

⇒ **Hybridizing done** is displayed when complete **(Figure A.29)**. The experiment is ready to be scanned.

83. Click each module to verify that the hybridization is complete.
84. Click **Close** to exit the Fluidics station window.
85. For TestExp_2, select the GeneChip Software shortcut bar and click the **Workflow Monitor** icon .

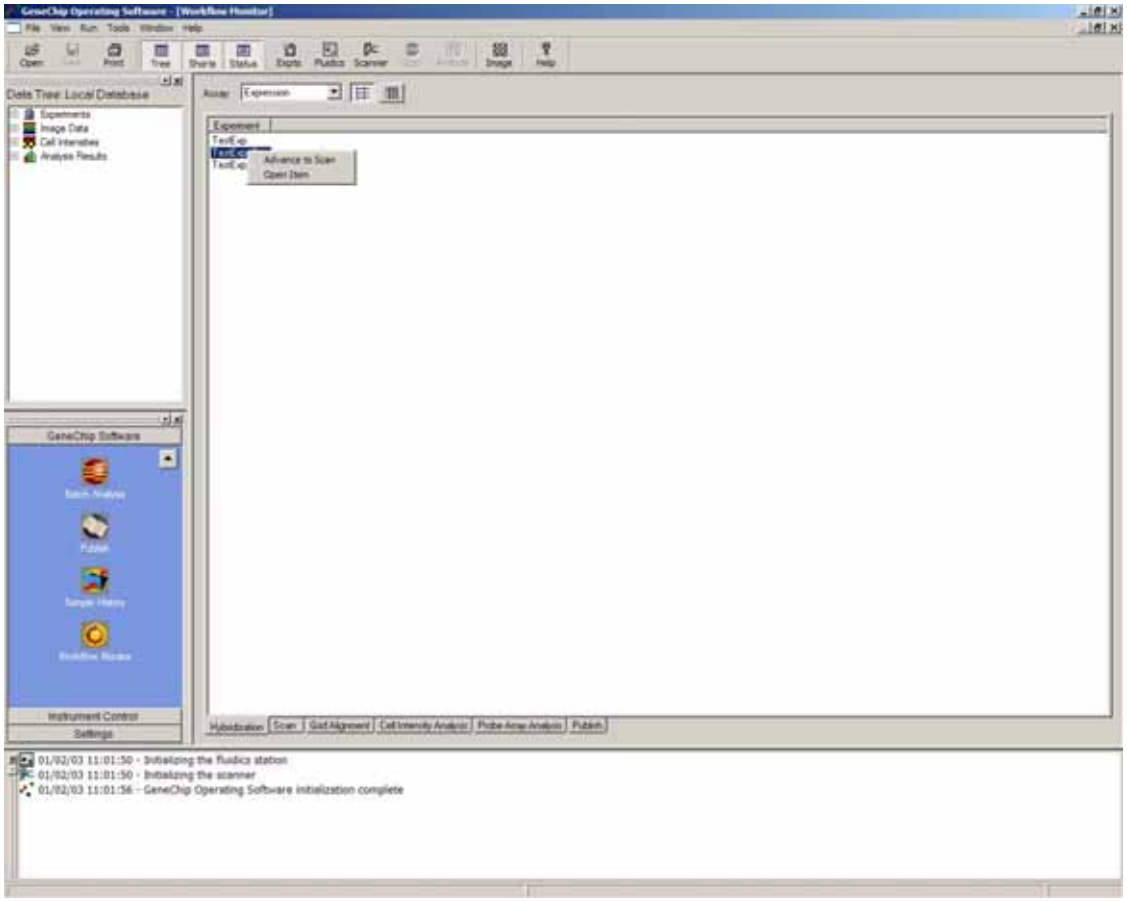


Figure A.30
Workflow monitor window

86. If you want to skip the hybridization step and place the experiment in scan status, right-click TestExp_2 and select **Advance to Scan** from the shortcut menu that appears **(Figure A.30)**.

Scanning an Experiment

87. To scan an experiment, select **Run →Start Scanner** from the menu bar, or click **Instrument Control** in the Instrument Control shortcut bar and then the **Start Scanner** button .

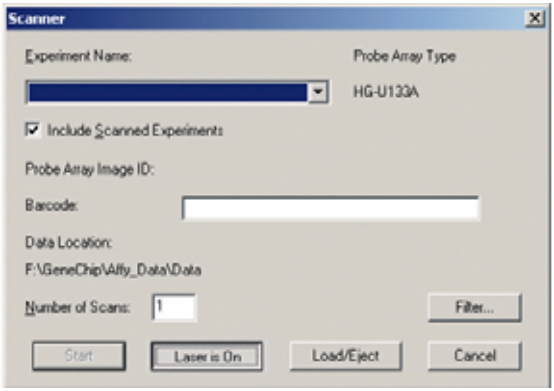


Figure A.31
Scanner dialog box

88. Select TestExp the **Experiment Name** drop-down list.

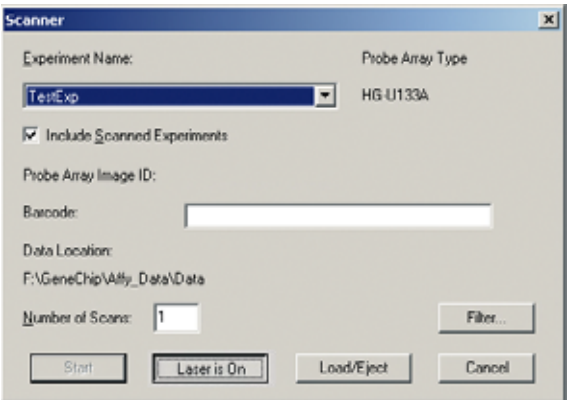


Figure A.32
Scanner dialog box, TestExp selected for scan

89. Click **Start** to begin scanning the chip.

Figure A.33
Scanner prompt

Note

Scanning a chip through one scan takes approximately 5 minutes. Only one chip can be scanned at a time.

90. Load the chip into the scanner.
    ⇒ After the scan is completed, GCOS generates a .cel.
91. Scan the second experiment: from the menu bar select **Run →Start Scanner** or click the Instrument control shortcut bar and click the **Start Scanner** button .
92. From the **Experiment Name** drop-down list, select **TestExp_2**.
93. Click **Start** to begin scanning the probe array.
    After the scan is completed, this experiment has the program automatically create the .cel and the .chp and publishes them into the MyPub database. This is done because **Analysis Set** and **Publish Database** fields were selected during the experiment setup.
94. Do not scan **TestExp_3**.

Running an Analysis

95. To generate a .chp, select the TestExp.CEL.
96. Right-click TestExp.CEL file and select **Analyze** **(Figure A.34)**.

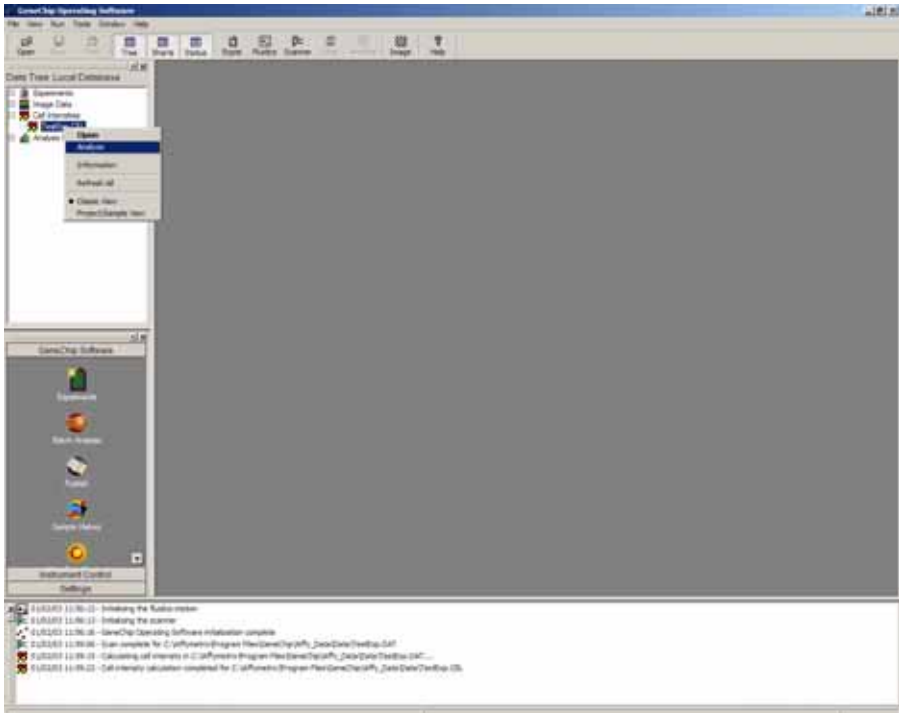


Figure A.34
Analyze Experiment window

⇒ The status log displays that it is running an analysis for the file. For example, Running analysis for \\<servername>\GeneChip\Affy_Data\Data\TestExp.CHP **(Figure A.35)**.

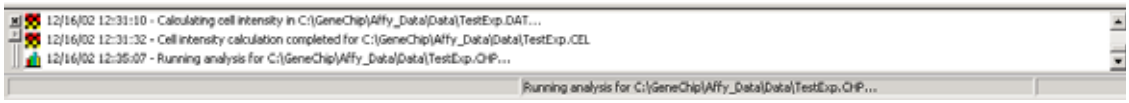


Figure A.35
Status log

97. The status log displays when the analysis is completed (i.e. Analysis completed for \\<servername>\GeneChip\Affy_Data\Data\TestExp.CHP). The .chp file is displayed.

    If an error message is displayed, make a note of the error message and follow the suggestions in the error message. If problems persist, refer to *GCOS Manager 1.0 Troubleshooting* on page 640.

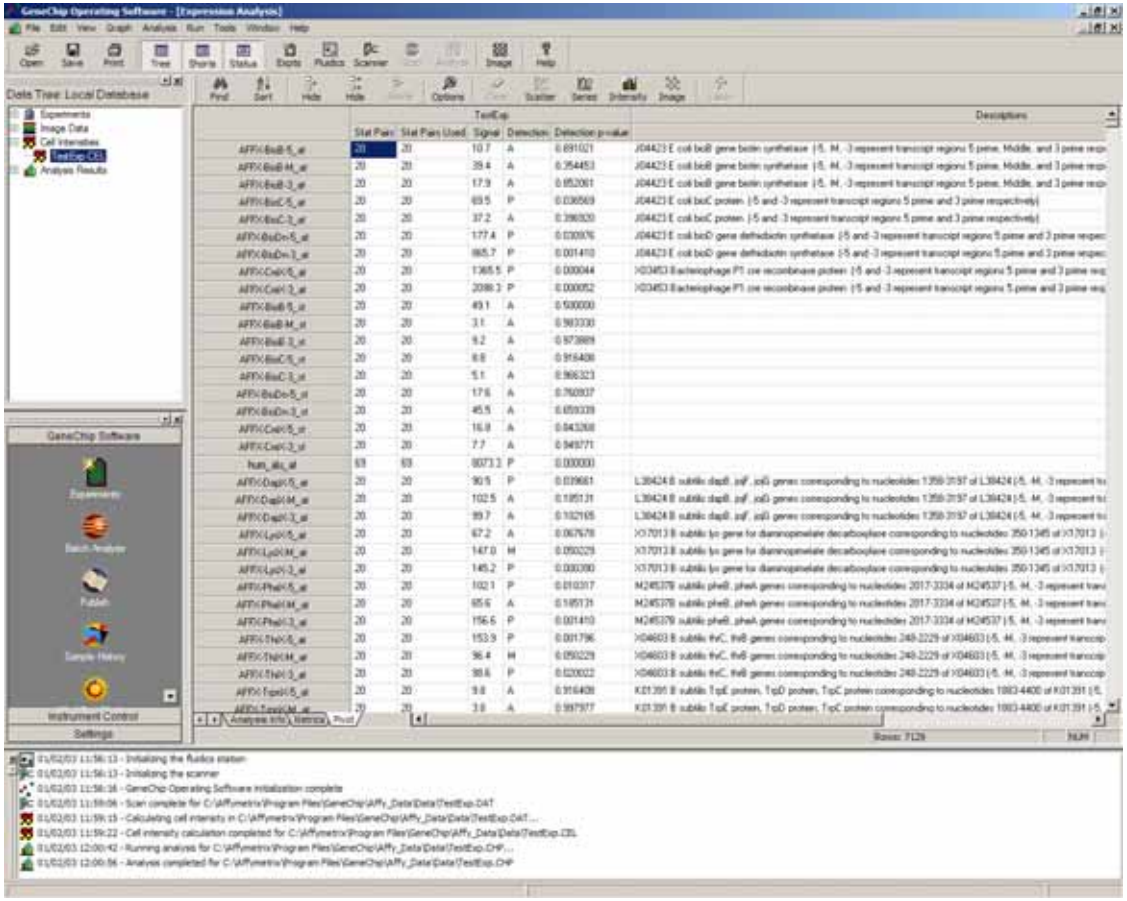


Figure A.36
Analysis output file (.chp) displayed

Publishing

98. To Publish an experiment, select **Run →Publish** from the menu bar or click the **Publish** button in the GeneChip Software shortcut bar .
    ⇒ The Publish window appears **(Figure A.37)**.

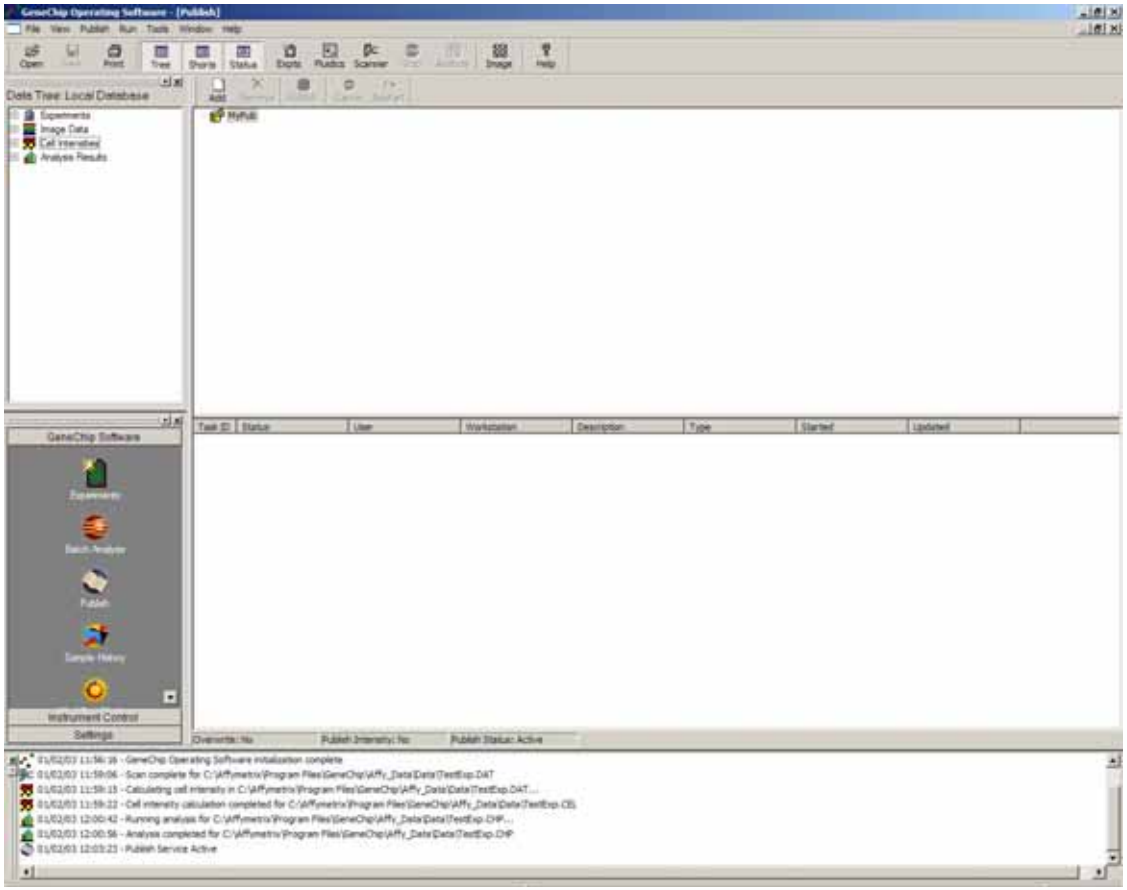


Figure A.37
Publish window

99. To add data to be published, select **Publish →Add Item** from the menu bar or click the **Add** button in the publish window area.
    ⇒ The Open window appears and displays experiments available for publishing **(Figure A.38)**.

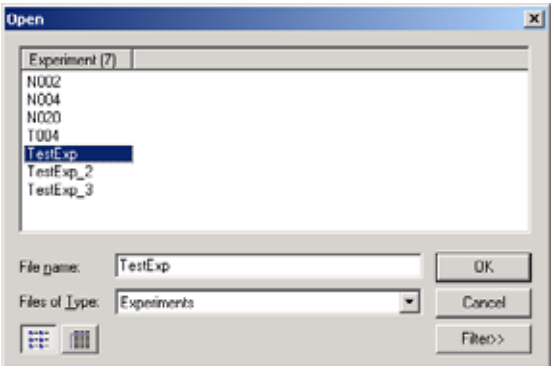


Figure A.38
Open window

100. Highlight the **TestExp** experiment and click **OK**.

101. To publish the cell intensity data, select **Publish →Options → Publish Intensities** from the menu bar.
    ⇒ The Publish Database Login window appears.

102. Enter the password for the publish database and click **Login**.

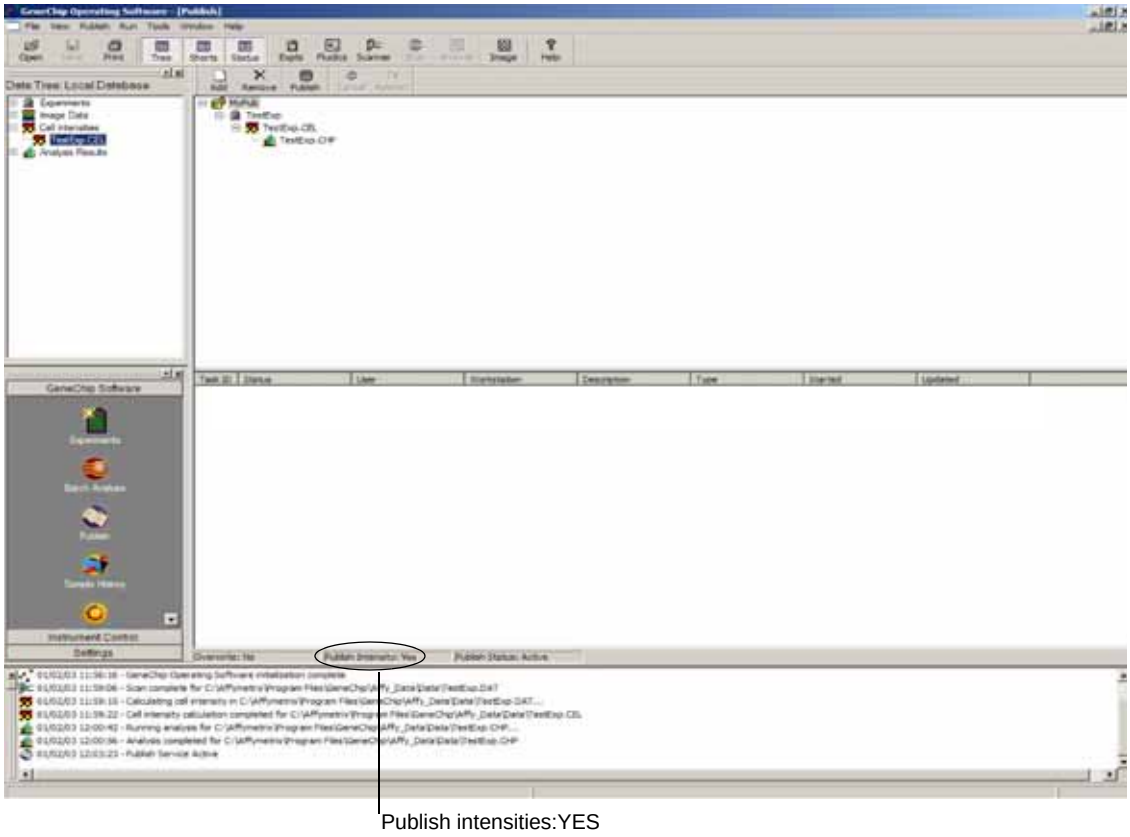


Figure A.39
Publish window with intensities selected

103. To begin the publishing process, select **Publish** →**Publish** from the menu bar or click the **Publish** button in the publish window.

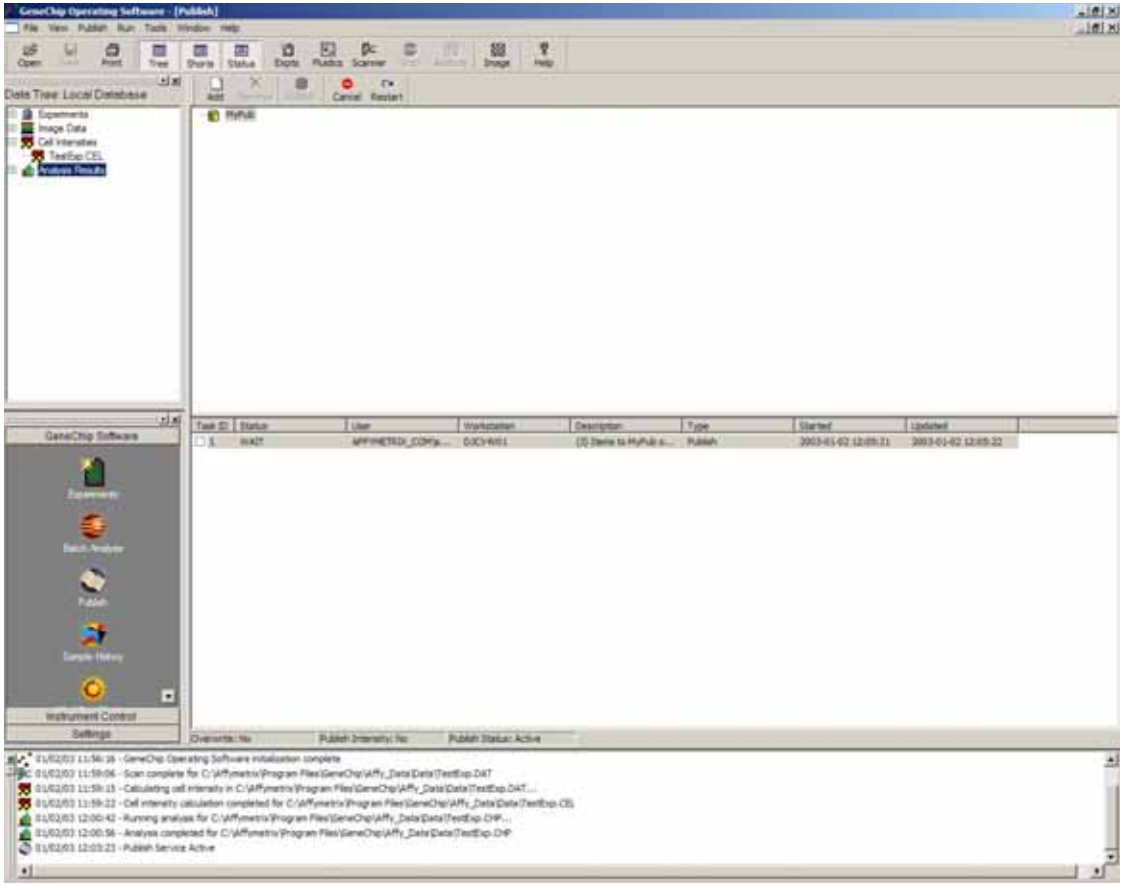


Figure A.40
Publish status window

104. The task window shows the status of the publishing process. Each task contains items that are in a particular task.

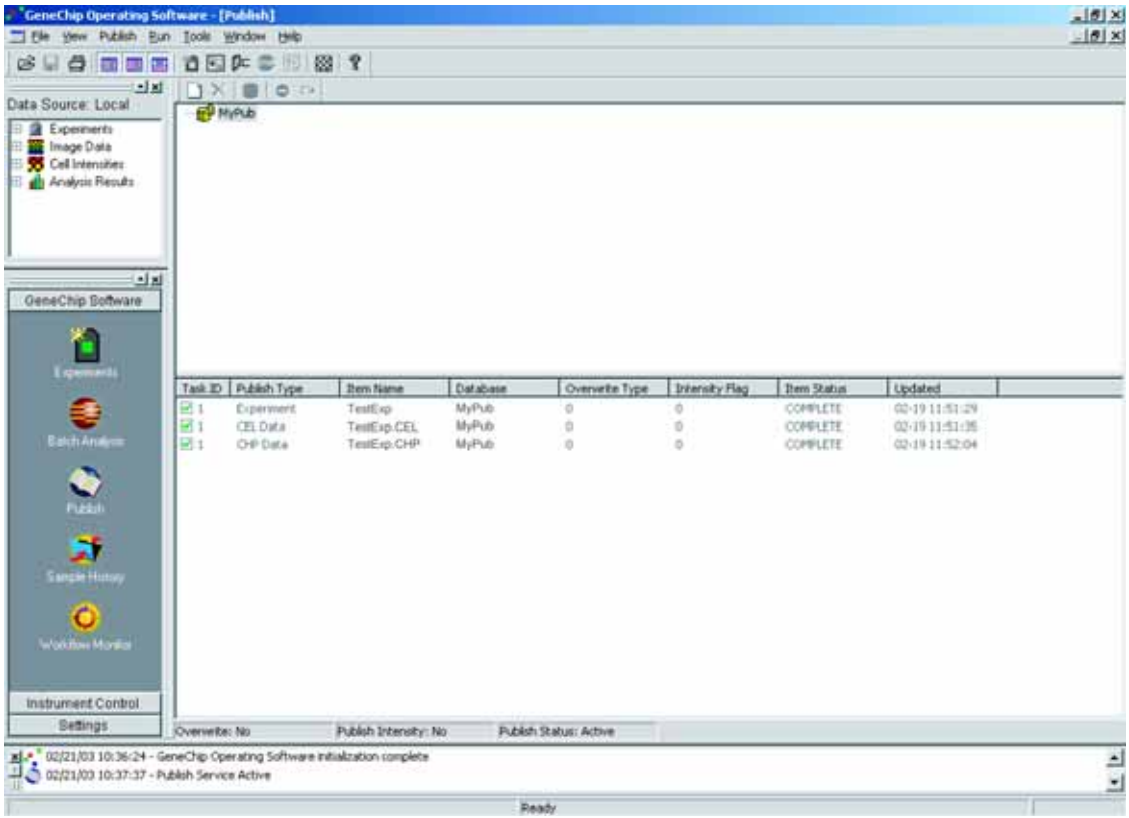


Figure A.41
Publish task item window

Running Reports

105. To configure the report settings, select **Tools** →**Report Settings** → **Expression Report f**rom the menu bar, or click the **Report Settings** in the shortcut bar and click **Expression Report** .

⇒ The Expression Report window appears **(Figure A.42)**.

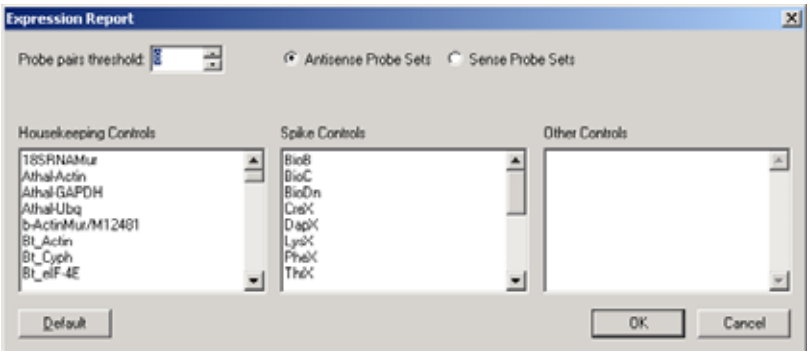


Figure A.42
Expression Report window

106. Select **File →Report** from the menu bar and select the .chp to generate the report. Alternatively, right-click a .chp in the data file tree, and select **Report** (use the **TestExp.CHP** file).

Sample History

107. To view the sample history window **(Figure A.43)**, select **Run →Sample History** from the menu bar, or click the **Sample History** button in the shortcut bar.

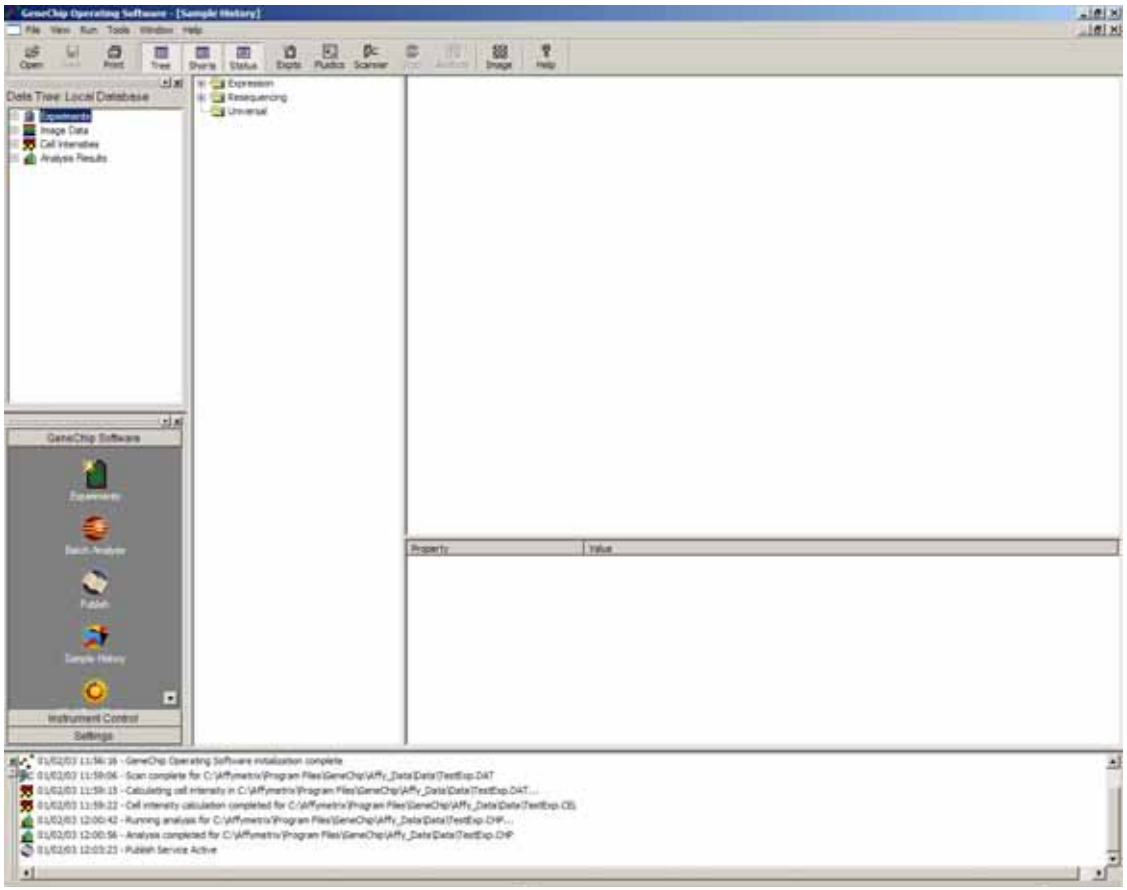


Figure A.43
Sample history window

108. Click a sample.

⇒ The list of files pertaining to the sample appears in the right hand pane **(Figure A.44)**.

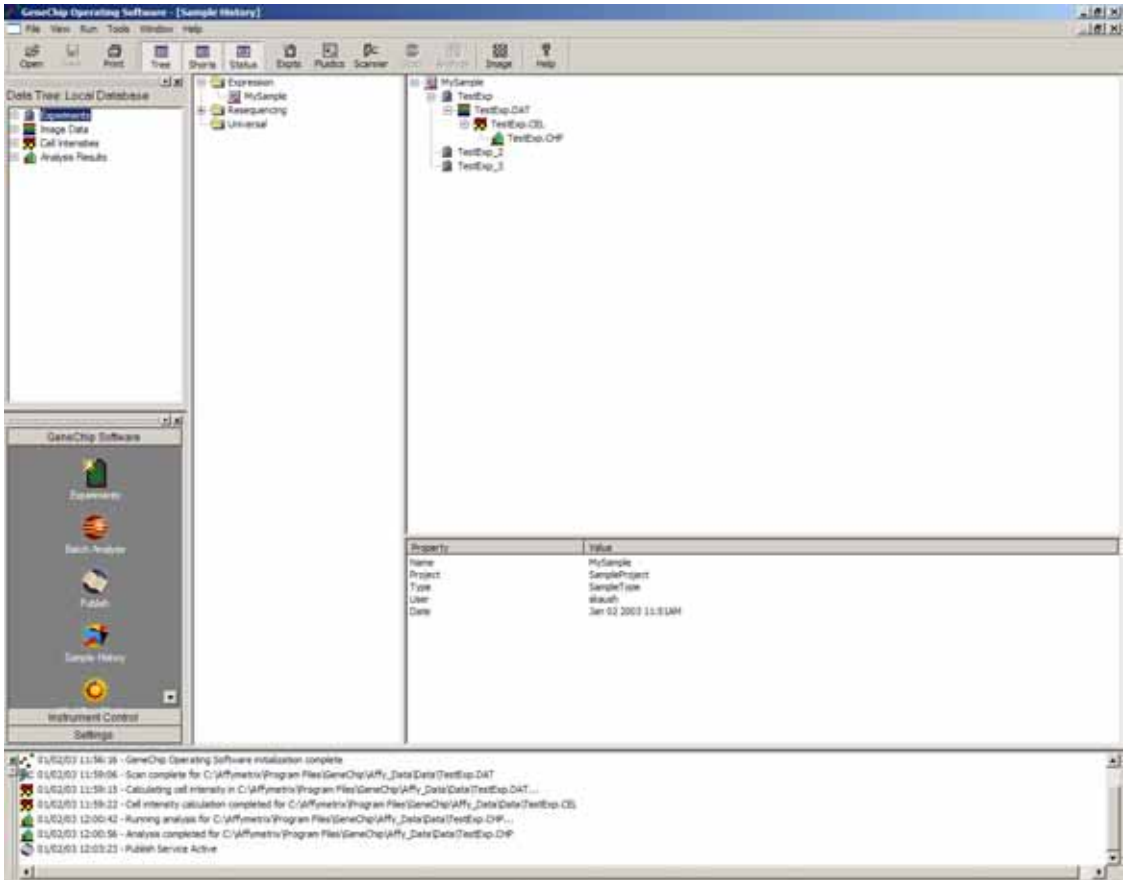


Figure A.44
Sample history window, selected file

109. To view more history information, click the **EXP** icon and information in the results window is displayed **(Figure A.25)**.

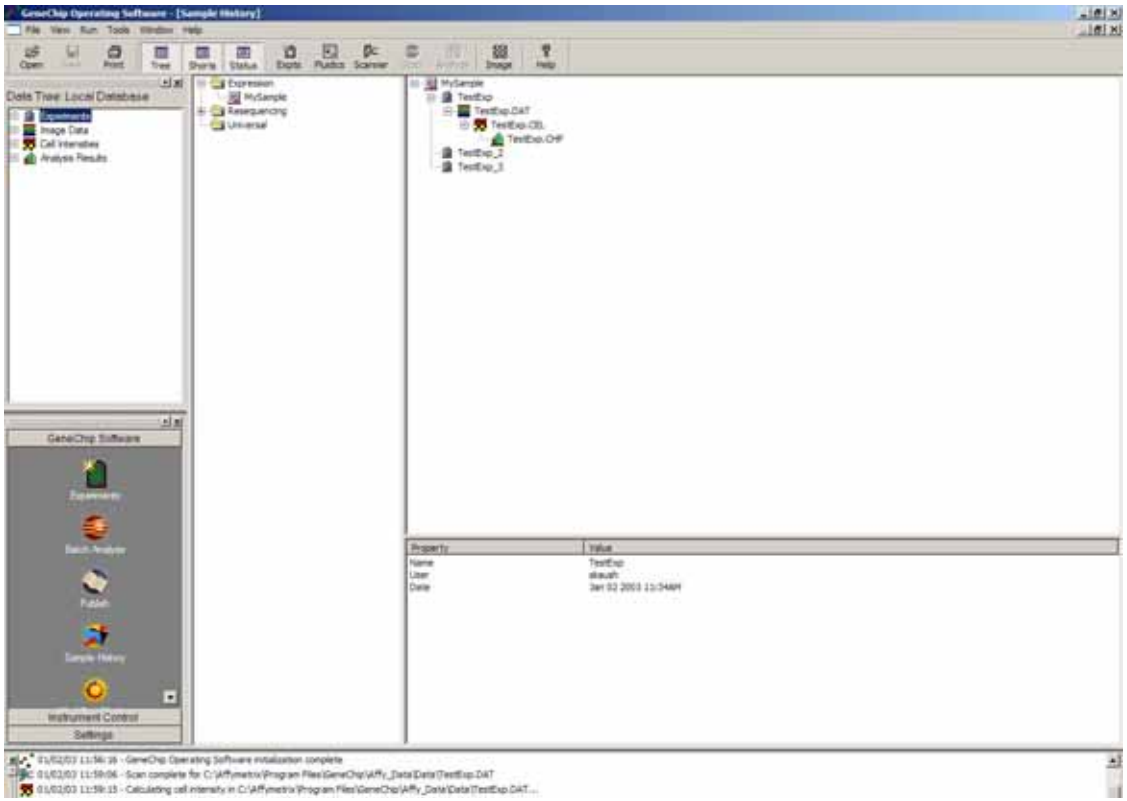


Figure A.45
History for the selected file

110. To view history information, click the .dat, .cel, or .chp, click the file.

⇒ The history information is displayed in the bottom windowpane.

Workflow Monitor

111. To view the workflow monitor, select **Run →Workflow Monitor** from the menu bar or click the **Workflow Monitor** icon in the GeneChip Software shortcut bar.

112. To filter information, select **Tools →Filters…** from the menu bar to display the Filters window.
113. Information can be filtered on the following items:
    - Assay Type
    - Sample Name
    - Experiment
    - Sample Project
    - Probe Array Type
    - Sample Type
    - User
    - Date
114. Click **OK** after any filter selections have been made.
115. The Workflow Monitor shows the current stage of the experiment. The experiment could be in the following stage status:
    - Experiment Setup
    - Hybridization
    - Scan
    - Grid Alignment
    - Cell Intensity Analysis
    - Probe Array Analysis
116. Click the bottom tabs to see the stage where experiments currently stand.

Data Mining Tool 3.1 Testing

1. Is your GCOS server using a SQL Server or Oracle database?
2. If using SQL Server, go to step 5 If using Oracle, go to step 3
3. If using Oracle, verify that Oracle client utilities are installed.
4. Verify that the alias or aliases (if using a remote GCOS server) are created.

✔ Note

*When you open the Data Mining Tool (DMT) for the first time a message appears stating "Unable to Determine Publish Database Name". Click **OK**. This message appears because a publish database has not yet been registered.*

5. Start Data Mining Tool (**Start →Programs →Affymetrix →Data Mining Tool**).
6. Select **Edit →Register Database** from the menu bar.
   ⇒ The Register Database dialog box appears **(Figure A.46)**.

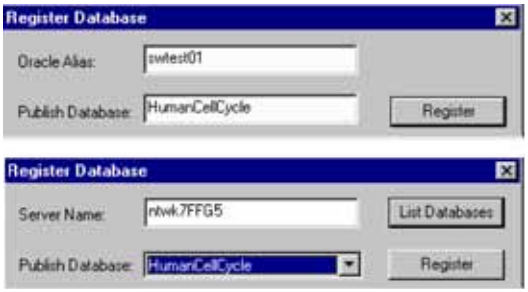


Figure A.46
Register Database dialog box, publish database on GCOS server (Oracle® dialog box, top; Microsoft® SQL Server dialog box, bottom)

7. If using an Oracle database, go to step 8. If using a Microsoft® SQL Server database go to step 10.
8. Enter the server's alias name in the **Oracle Alias** box.

9. Enter the **Publish Database** name, then go to step 12.
10. Enter the **Server Name**, then click **List Databases** to display the publish databases for the server in the **Publish Database** drop-down list.
11. Select a database from the **Publish Database** drop-down list.
12. Click **Register**.
    ⇒ DMT prompts for the Publish Database Password.
13. Enter the **Password** and click **Login**.
    ⇒ The database is available to DMT.
14. Select **Edit →Select Data Source** from the menu bar.
15. Select the Oracle Publish DSN **(MyPub**, or the name of the DSN created).
16. Select **Data →New →Expression Data Mining Tool** from the menu bar.

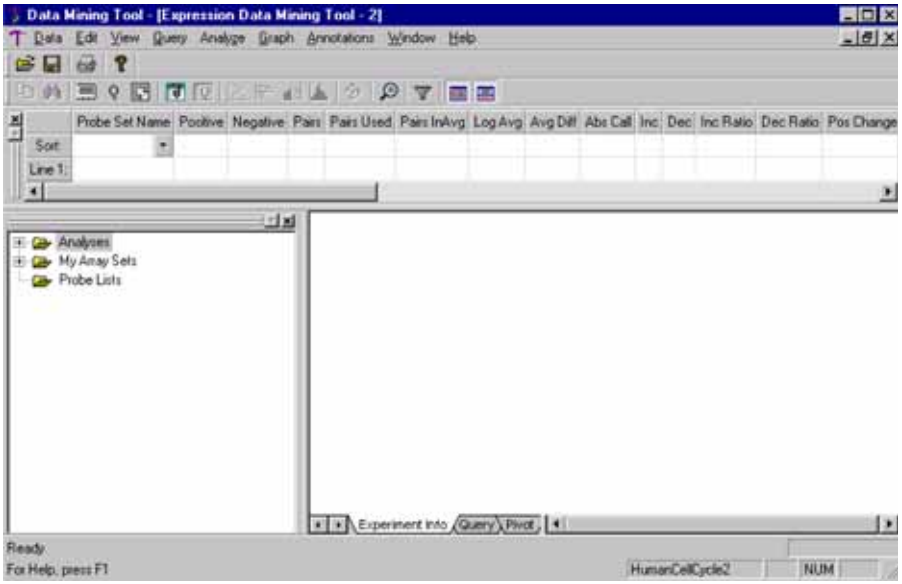


Figure A.47
Data Mining Tool window

17. Verify that the data published appears in the DMT window.
18. From the menu bar, select **Query →Run Query** to execute query or click the **Query** icon Q.
    ⇒ The query results window is displayed **(Figure A.48)**.

	Analysis Name	Probe Set Name	Positive	Negative	Pairs	Pairs Used	Pairs InAvg	Pos Fraction	Log Avg	Pos/Neg	Avg Diff	Norm Avg Diff	Abs Call
1	N002	AFFX-BioC-5_at	7	1	20	20	18	0.35	1.67	7.0	87.1	259.1	P
2	N002	AFFX-BioDn-5_at	9	3	20	20	20	0.45	1.75	3.0	173.1	514.8	P
3	N002	AFFX-BioDn-3_at	14	2	20	20	19	0.70	3.22	7.0	811.0	2412.6	P
4	N002	AFFX-CreX-5_at	19	0	20	20	19	0.95	6.64		864.8	2572.7	P
5	N002	AFFX-CreX-3_at	19	0	20	20	19	0.95	6.59		1591.7	4735.1	P
6	N002	hum_alu_at	69	0	69	69	69	1.00	6.15		6375.6	18966.9	P
7	N002	AFFX-DapX-5_at	14	0	20	20	19	0.70	2.95		491.3	1461.7	P
8	N002	AFFX-DapX-M_a	14	1	20	20	17	0.70	3.15	14.0	377.3	1122.5	P
9	N002	AFFX-DapX-3_at	17	1	20	20	19	0.85	4.02	17.0	467.7	1391.5	P
10	N002	AFFX-LysX-5_at	14	0	20	20	19	0.70	3.77		319.3	949.9	P
11	N002	AFFX-LysX-M_at	17	0	20	20	20	0.85	4.55		736.3	2190.3	P
12	N002	AFFX-LysX-3_at	15	0	20	20	20	0.75	4.71		794.9	2364.9	P
13	N002	AFFX-PheX-5_at	17	0	20	20	19	0.85	4.63		472.1	1404.3	P
14	N002	AFFX-PheX-M_at	15	0	20	20	19	0.75	3.40		454.6	1352.5	P
15	N002	AFFX-PheX-3_at	17	0	20	20	18	0.85	3.86		599.1	1782.4	P

Experiment Info | Query | Pivot

Figure A.48
Query Results window

19. Pivot the query results data by selecting **Query →Run Pivot** from the menu bar or click the **Pivot** button.

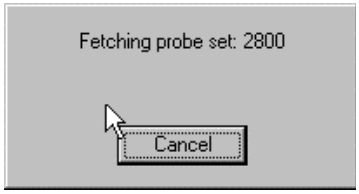


Figure A.49
Pivot running

20. Graph the results by selecting **Graph →Scatter...** from the menu bar or click the **Scatter Graph** button.

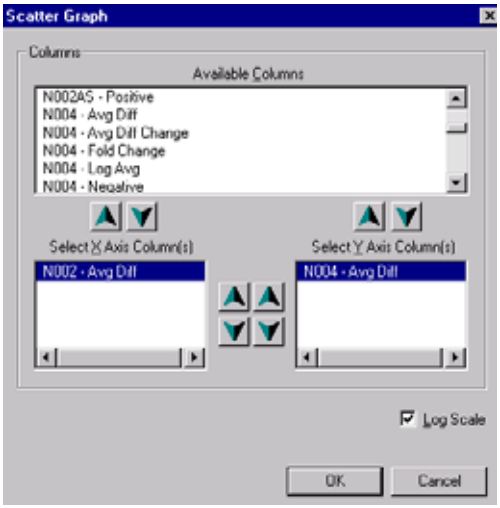


Figure A.50
Scatter Graph dialog box

21. Move the analysis files to the X-Axis and the Y-Axis. Click **OK**

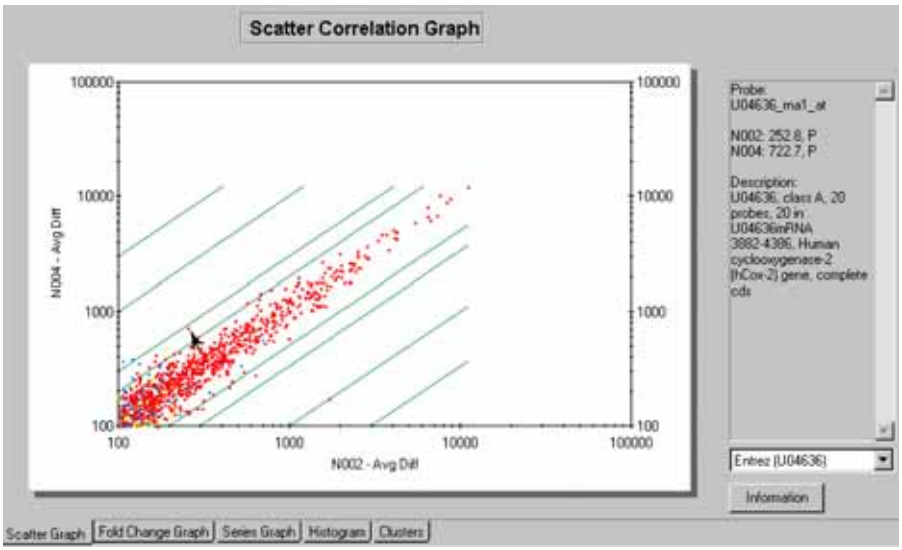


Figure A.51
Scatter Graph

GCOS Manager Additional Testing

This additional testing allows the system administrator to delete, archive, unarchive, export or take ownership of data.

22. Start GCOS Manager 1.0 (**Start →Programs →Affymetrix →GCOS Manager**).
23. Expand the **Samples** list.
24. Expand the **Expression Sample** list.

Export an Experiment

25. Select **Tools →File Path** from the menu bar, or click the **File Path** button .
26. Select a path location to export the files to (for example, x:\temp).
27. Click the sample named MySample and select **Process →File View**.
28. In the right hand pane, select **TestExp** and right-click to select **Export,** or go to the menu bar and select **Process →Export** after selecting the **TestExp** **(Figure A.52)**.

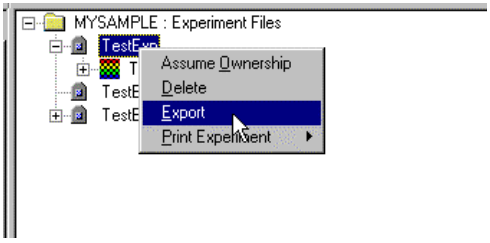


Figure A.52
Export window

⇒ The task window displays the export process. It exports the .dat, .cel, and .chp **(Figure A.53)**.

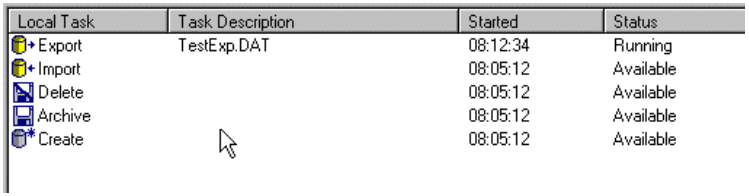


Figure A.53
Status window

29. Once completed, the status returns to Available. If it fails, the status returns 'Failed.'
30. To view the error, double-click the **Export** icon in the Local Task pane .

    ⇒ A text file appears. View the error message and try to resolve it. Try exporting again.

Archive and Unarchive

31. Click the Process Tab.
32. Expand the TestExp list in the right hand pane.
33. Select the TestExp.CEL file.
34. Right-click and select **Archive** **(Figure A.54)**, or select **Process →Archive** from the menu bar.

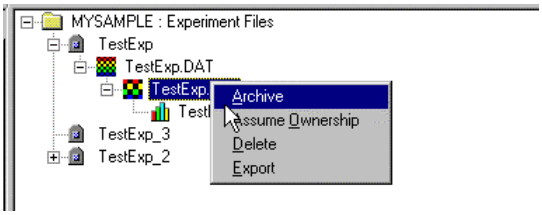


Figure A.54
Archive window

⇒ The Select Archive Path Location window appears **(Figure A.55)**.

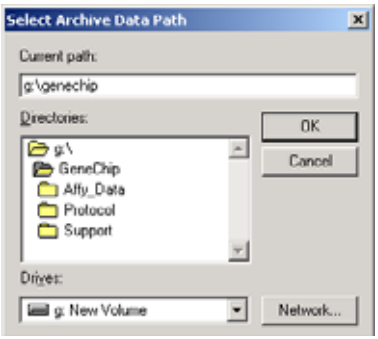


Figure A.55
Archive Path Location window

35. Select the path location to archive the files to (for example, c:\temp). Click **OK**.

    ⇒ The archive process begins. All files are archived from the parent down. If there are any files below, it archives them as well. In this example, the .cel and .chp are archived.

    The archive process is completed when the status is Available.

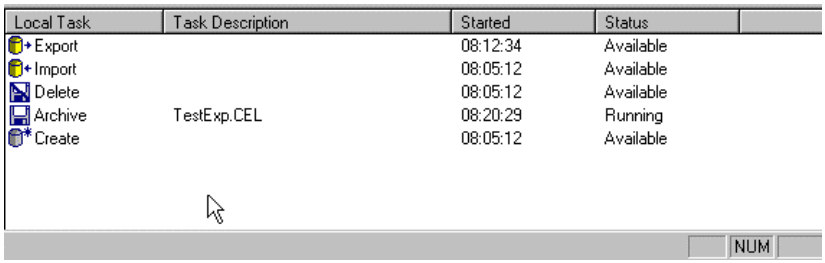


Figure A.56
Status window

36. Click MySample in the Samples list and select **Process →Refresh** from the menu bar.

37. Expand **TestExp** in the right hand pane.

    ⇒ The files are archived and are displayed in gray.

38. To unarchive, right-click the archived file and select **Unarchive**. Alternatively, click the archived file and select **Process →Unarchive** from the menu bar.

    ⇒ The **Unarchive** process begins.

Publish Database Data Deletion

39. To delete files from the Publish database, select the **Publish** tab.
40. Expand the **Probe Array Type** list.

    ⇒ A list of probe array types is displayed of published experiments.
41. Click **Hu6800**.

    ⇒ The published experiments is displayed.
42. Click **TestExp**.

    ⇒ This expands the list to display the .cel and .chp files **(Figure A.57)**.

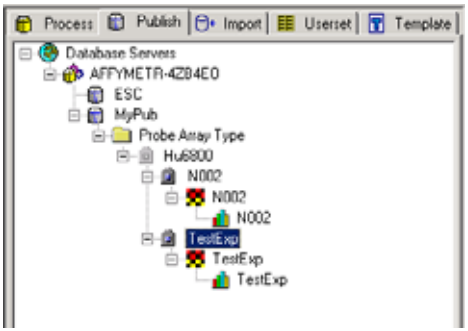


Figure A.57
Publish Tab

43. Select the TestExp.CHP file (the last file), right-click and select delete or select **Publish →Delete** from the menu bar.

    ⇒ The confirmation message to delete appears **(Figure A.58)**.

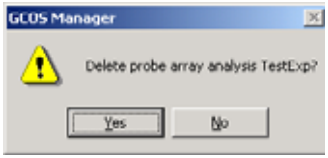


Figure A.58
Confirmation to delete selected Publish data

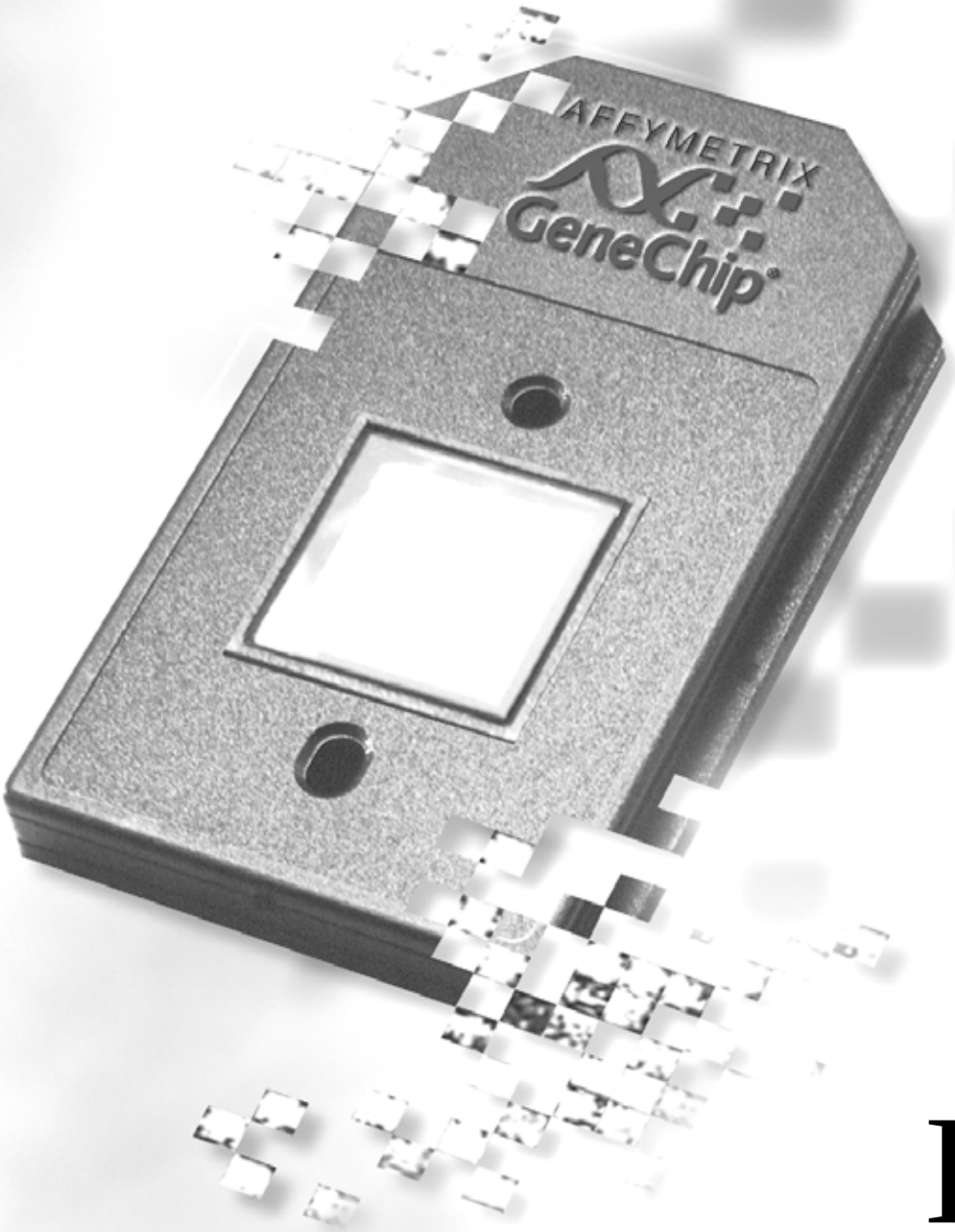
44. Click **Yes**.
    ⇒ A message appears informing that the file is being deleted. The deleted .chp is removed from the data tree.
45. If the experiment remains in the process database, the data can be republished.

Process Database Data Deletion

46. To delete data from the process database, select the **Process** tab.
47. Expand the Expression Samples list.
48. Expand MySample.
49. Right-click TestExp and select **Delete** from the shortcut menu that appears. Alternatively, click TestExp and select **Process →Delete** from the menu bar.
    ⇒ A confirmation window appears **(Figure A.59)**.

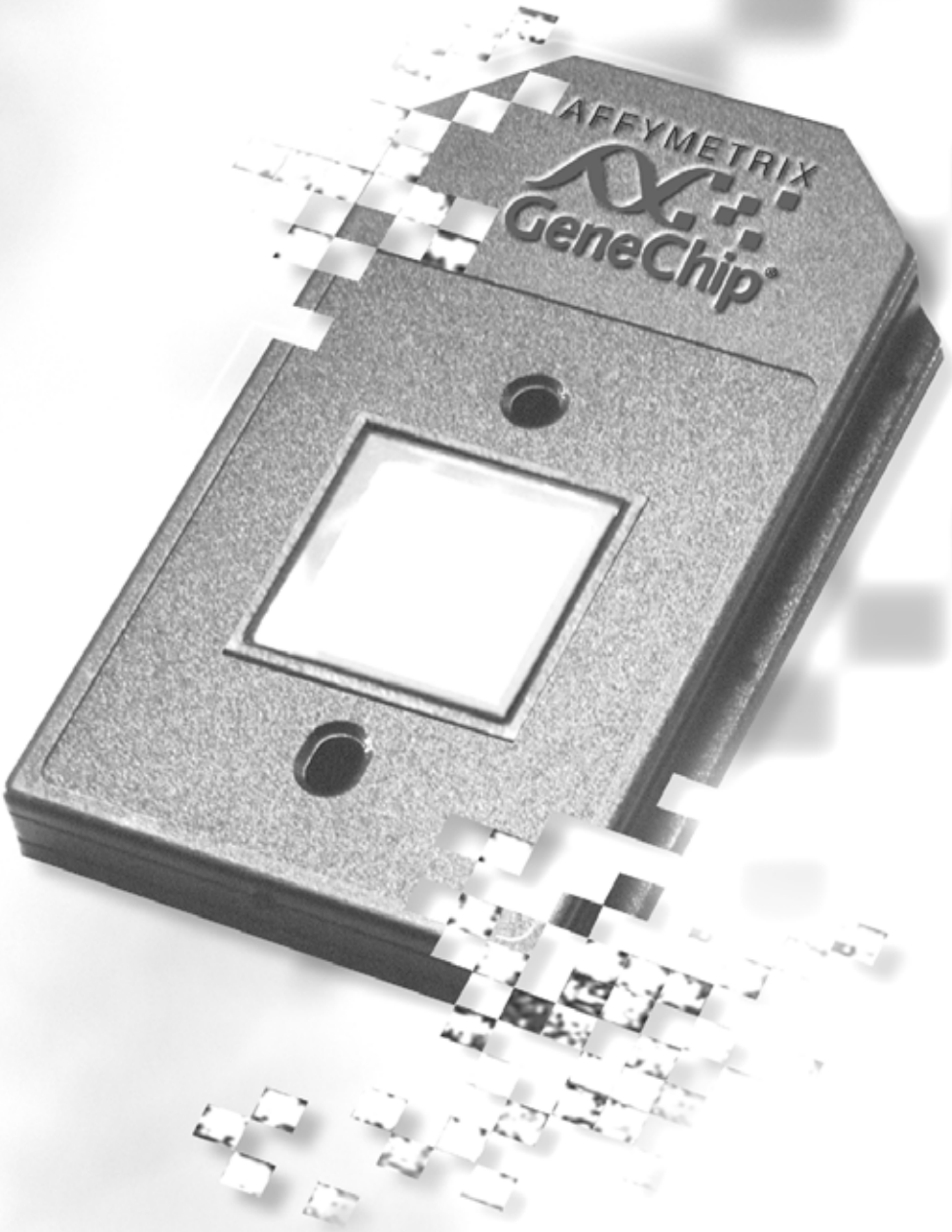
Figure A.59
Confirmation to delete specific experiment selected

50. Click **Yes**.
    ⇒ After deletion, the experiment is removed from the list.
51. Client testing completed.



B

Appendix B



Installing Data Mining Tool 3.1

The installation of Data Mining Tool 3.1 does not remove any previous versions of Data Mining Tool. If the DMT is installed in the same directory, it overwrites the previous versions' files.

Data Mining Tool 3.1

This section guides you through the installation of Data Mining Tool 3.1. Listed below is an overview of the steps needed to complete the installation:

Microsoft® SQL Server GCOS Server Users

1. Obtain the name of the GCOS server from your IT personnel if not known (this is needed during installation).
2. Install Data Mining Tool 3.1.

Oracle® GCOS Server Users

1. Install Oracle Client Utilities on the workstation (Oracle Client Utilities must be the same version installed on the GCOS server).
2. Install SQL* Loader (for better performance).
3. Create an Oracle Alias. Refer to *Creating an Oracle® Alias* on page 197.
4. Install Data Mining Tool 3.1.

The following are detailed installation instructions for installing DMT.

✔ NOTE

The screen captures depicted in this section may not exactly match the windows displayed on your screen.

You must be logged in as administrator to install the DMT 3.1 software.

1. Log in as an administrator.
2. Insert the Affymetrix® DMT 3.1 CD-ROM.
3. If the autorun feature does not start the program:
   a. Click **Start** →**Run**.
   b. Type <*cd drive letter*>:\setup.exe.
   c. Click **OK**.
   ⇒ The Affymetrix Software Setup window appears, followed by the Welcome window **(Figure B.1)**.

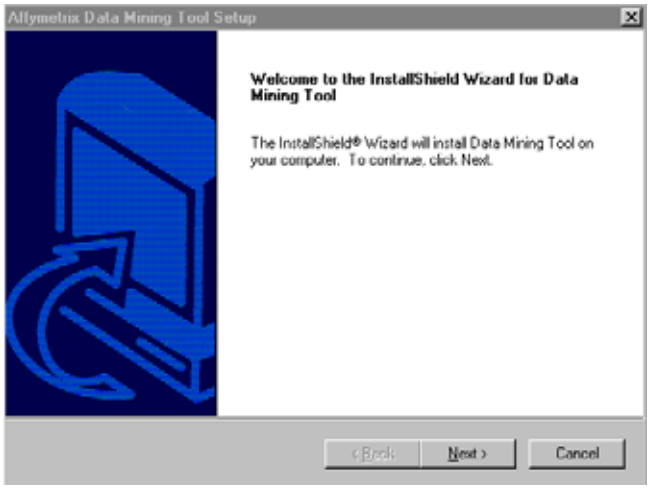


Figure B.1
Welcome window

4. Click **Next**.
5. Several consecutive Software License Agreement windows appear. Review the contents in each and click **Yes** to accept the terms of the agreement.
   ⇒ The Customer Information window appears **(Figure B.2)**.

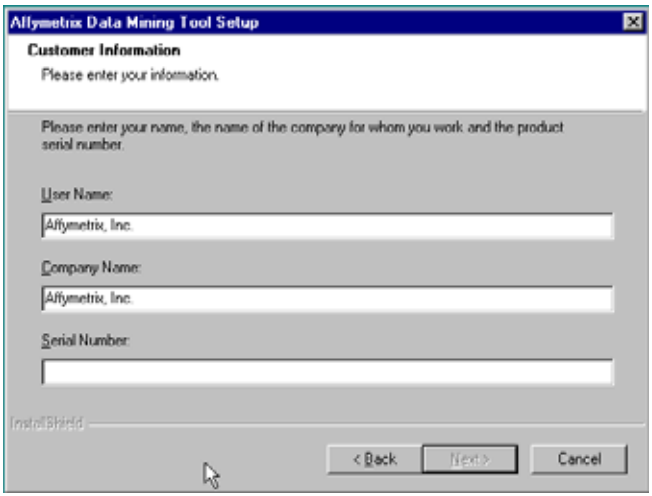


Figure B.2
Customer Information window

6. Enter your **Name**, **Company** and **Serial Number**.

   The serial number is located on the *Affymetrix® Software Product Registration* card.

✔ NOTE

If you do not have a serial number, contact Affymetrix Technical Support. If you are upgrading from a previous version, the Serial Number field populates automatically.

7. Click **Next**.

   ⇒ The Choose Destination Location window appears **(Figure B.3)**.

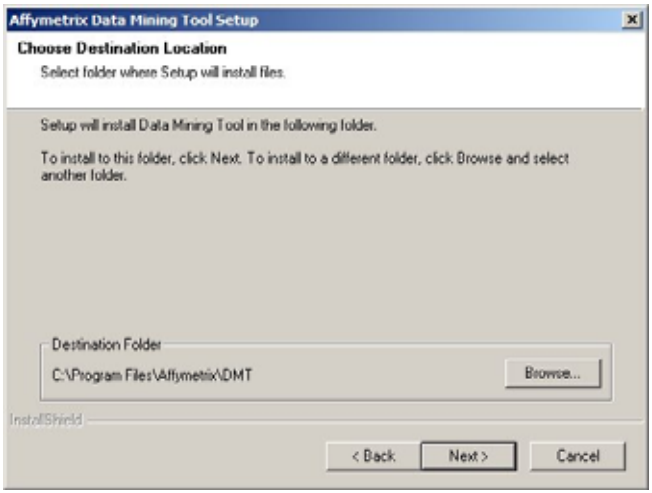


Figure B.3
Choose Destination Location window

8. Click **Next**.

   ⇒ The Select Database Compatibility window appears **(Figure B.4).**

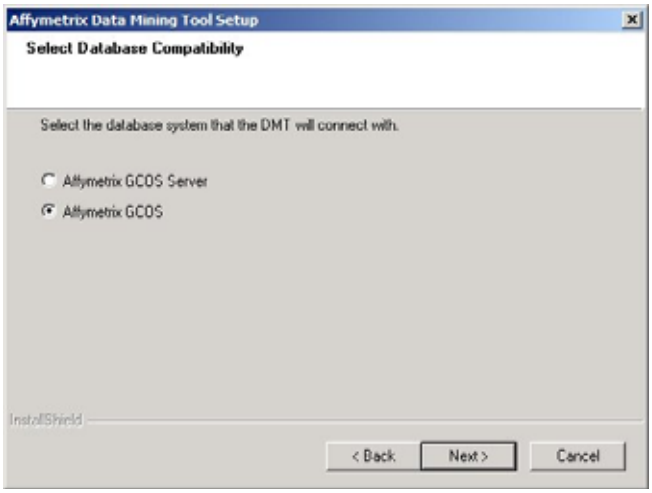


Figure B.4
Select Database Compatibility window

9. Select the database that DMT will connect with.
   - **Affymetrix® GCOS Server** - if connecting to a GCOS server
   - **Affymetrix® GCOS** - if connecting to the local MSDE database
10. Click **Next**.

    ⇒ The Select Database Type window appears **(Figure B.5)**.

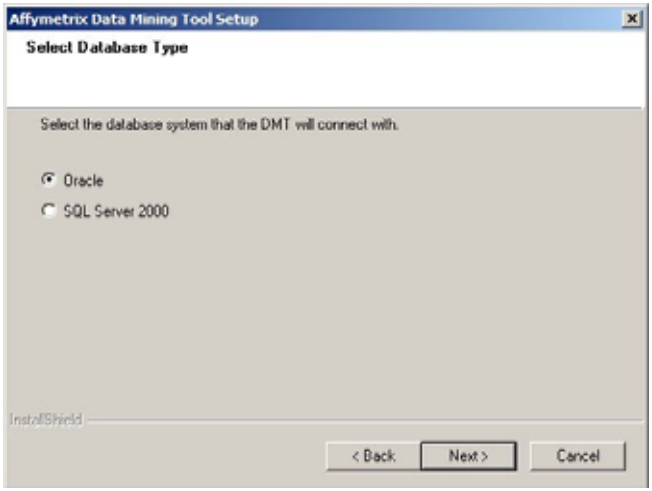


Figure B.5
Select Database Type window

11. Select the database used on the GCOS server, either **SQL Server** or **Oracle**.

    If you do not know the type of database you are using with the GCOS server, please contact your IT personnel or DBA.
12. Click **Next**.

    ⇒ The Enter Information window appears **(Figure B.6)**.

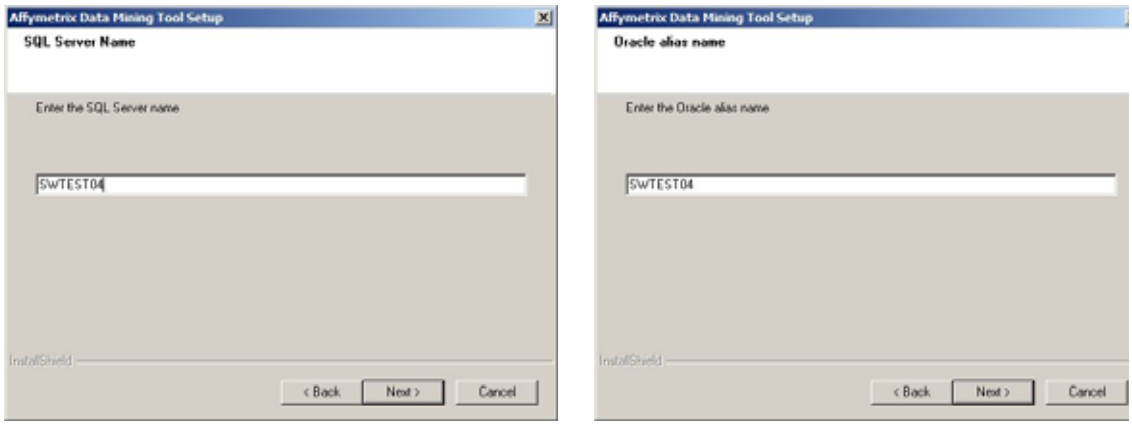


Figure B.6
Enter Information windows for the Microsoft® SQL Server database (left) or the Oracle® database (right)

13. In the Enter Information window complete one of the following;
    - If SQL Server is selected, enter the **SQL Server Name** (usually the name of the GCOS server).
    - If Oracle® is selected, enter the **Oracle Alias Name**.
14. Click **Next.**

    ⇒ The Start Copying Files window appears.
15. In the Start Copying Files window, verify the information and click **Next**.

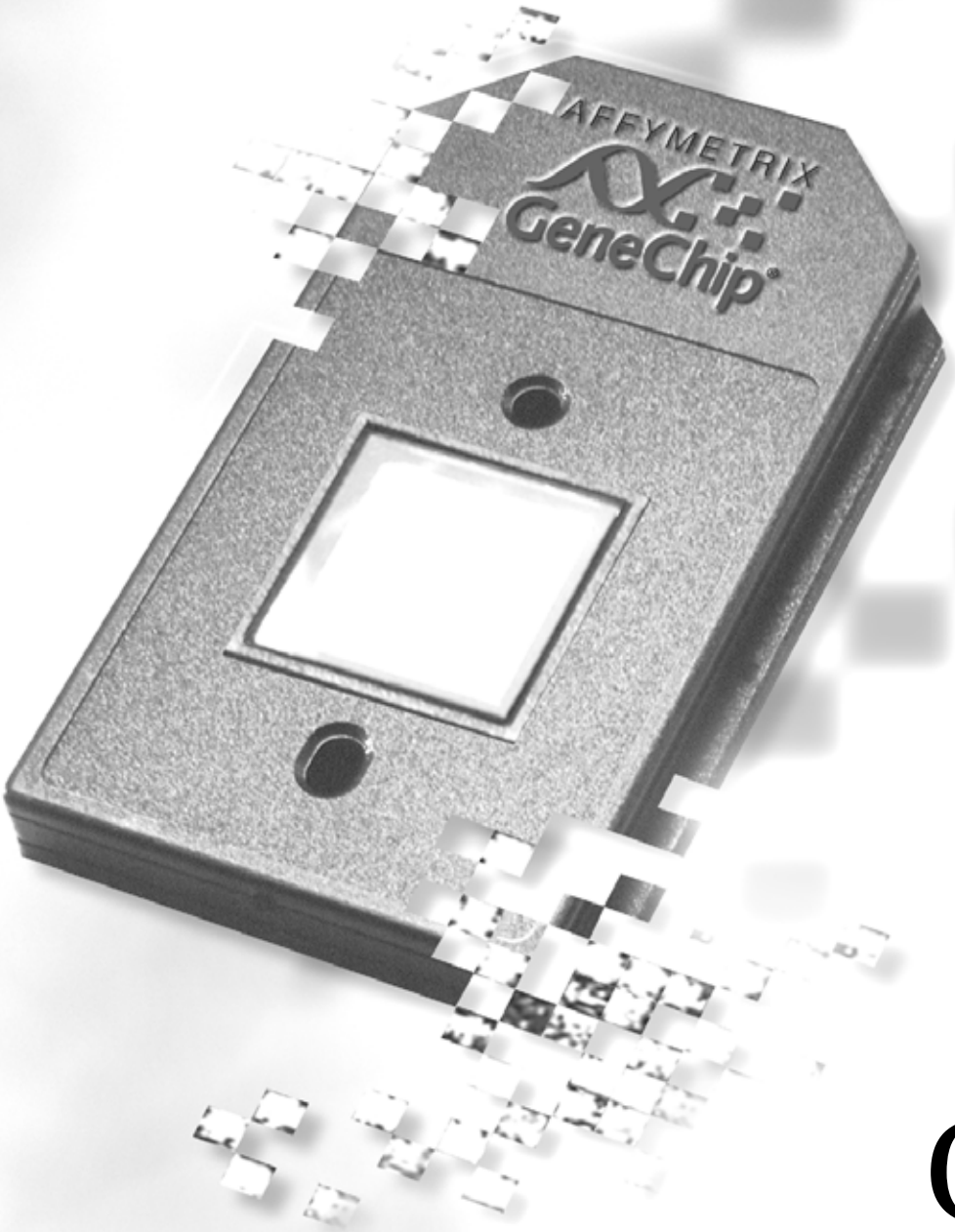
    ⇒ Program files are copied and the system configures the registry. Database connectivity is verified.

✔ NOTE

For Oracle systems: If a warning message regarding SQL Loader appears, continue the DMT install until complete. Then, install SQL Loader (part of Oracle) for better DMT performance. After SQL Loader is installed, re-install DMT.

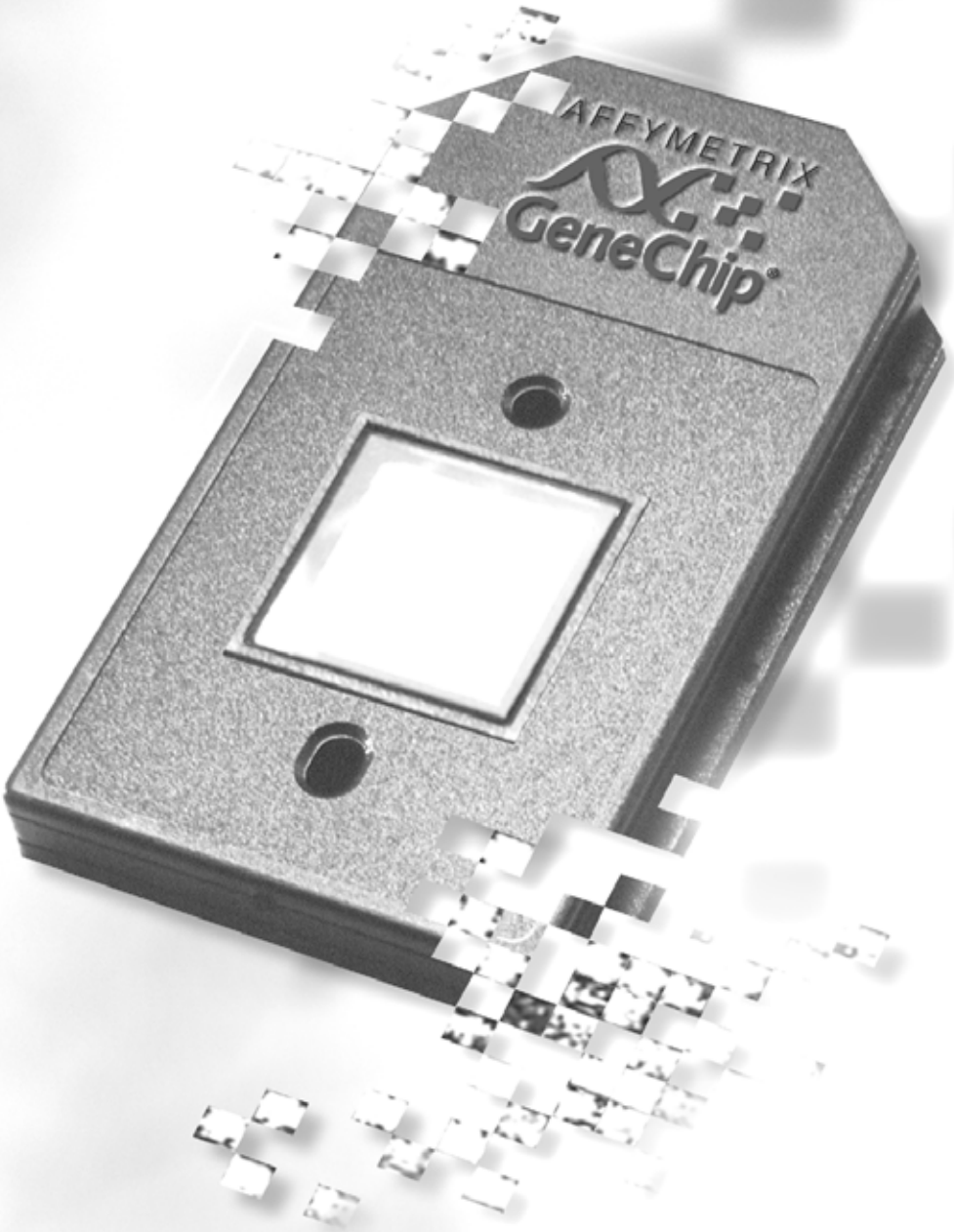
⇒ The Setup Complete window appears.

16. Select **Yes, I want to restart my computer now** and click **Finish**.



C

Appendix C



GCOS Instrument Installation

The instrument installation needs to be done only on an instrument system. Appendix C sections are:

- Fluidics Station and Scanner Installation
- Fluidics Station Installation (only)
- GeneArray® Scanner Installation (only)
- GeneChip® Scanner 3000 Installation (only)

The instrument installation needs to be run if there has been a change to any of the drivers, otherwise upgrading the software to GCOS is the only installation upgrade required.

✔ NOTE *The instrument installation is for Microsoft® Windows 2000 Workstation only.*

Scanner with Fluidics Station Installation

GeneArray® 2500 Scanner and Fluidics Station Installation

Follow this section if you have the GeneArray® 2500 scanner.

When you install the fluidics station software, confirm the presence of the correct Sealevel card.

✔ NOTE *If you cannot confirm the presence of a Sealevel card, contact Affymetrix technical support.*

1. On an instrument workstation that will be controlling fluidics stations, there must be a Sealevel card for communication between the

workstation and the fluidics stations. Review **Figure C.1** to confirm that a Sealevel card is installed in your system.

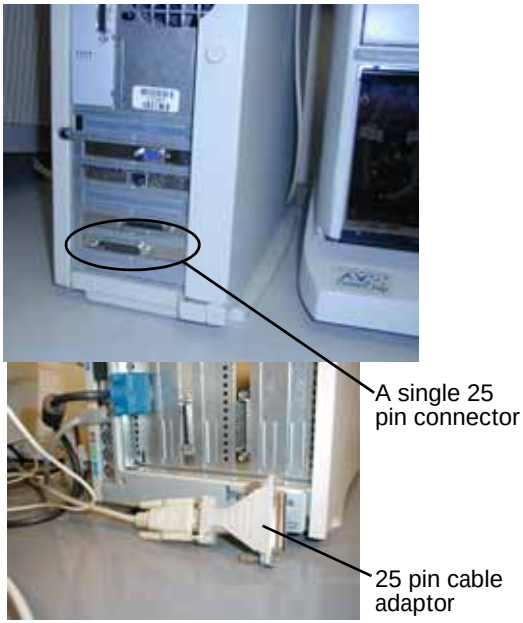


Figure C.1
Confirming the Sealevel card setup: note the 25 pin connector and the 25 pin cable adaptor

2. Launch **Windows 2000 Explorer**.
3. Browse to the Instrument CD.
4. Double-click setup.exe within the Instrument CD.

   ⇒ The **Welcome** window appears.
5. Click **Next**.
6. Several consecutive **Software License Agreement** windows appear. Click **Yes** in each window to accept the terms of the agreement.

   ⇒ The Choose Destination Location window appears **(Figure C.2)**.

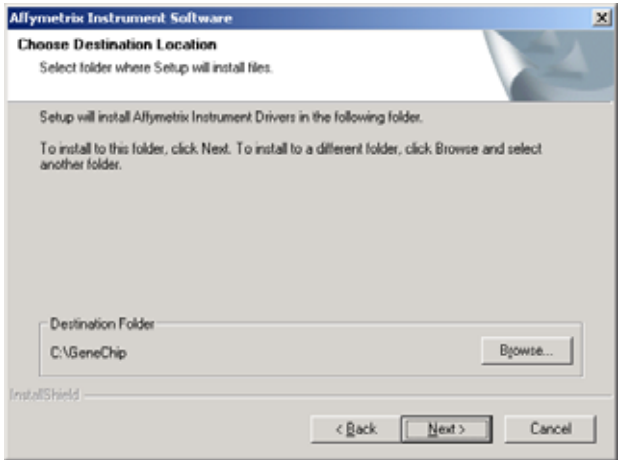


Figure C.2
Choose Destination Location window

7. Click Browse and select the **Destination** to install the instrument driver (select the **same location** where you installed Microarray Suite).

8. Click **Next**.

   ⇒ The Select Components window appears **(Figure C.3)**.

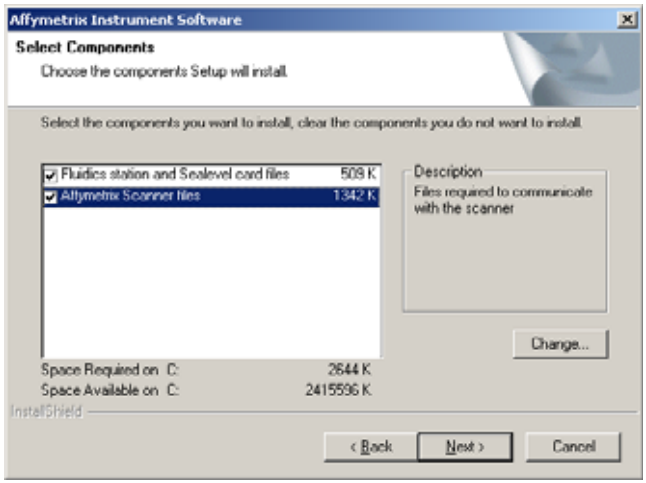


Figure C.3
Select both the Scanner Files and Fluidics Station Files and Sealevel card files

9. Select both the **Affymetrix Scanner Files** option and the **Fluidics Station Files** option **(Figure C.3)**. Highlight the **Affymetrix Scanner Files** option and click **Change...** to select the type of scanner.

   ⇒ The following dialog box opens and displays the choice of scanners, Affymetrix® or GeneArray® **(Figure C.4)**.

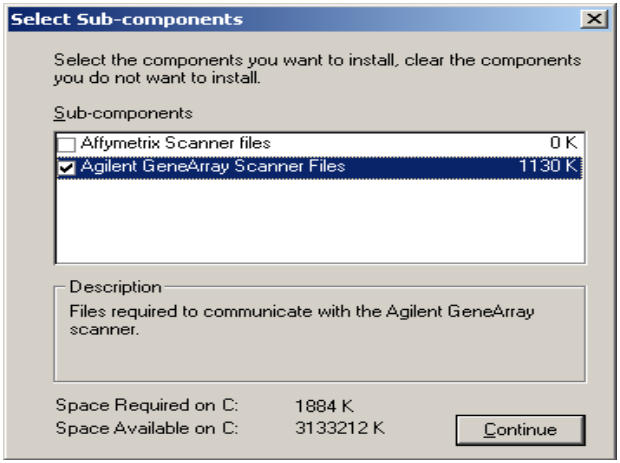
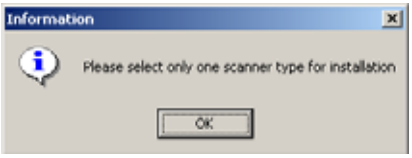


Figure C.4
Select the GeneArray® scanner. Select one scanner but not both.

10. Select the GeneArray® 2500 scanner Files and click **Continue**.

    If you click **Continue** with both scanners selected, the software will display the following message.



11. Click **Next** and enter the Port # for the COM serial port for the Sealevel serial card. Enter **2** **(Figure C.5)**.

✔ NOTE *If the workstation is the GX110, GX260, or GX270, select COM Port 3.*

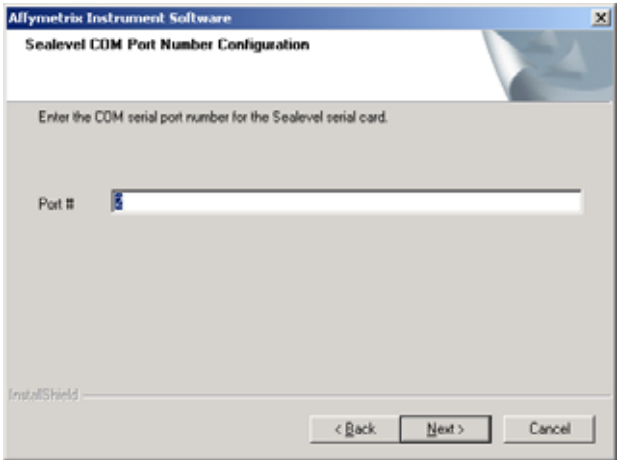


Figure C.5
Select the Port Number (Sealevel card only)

12. Click **Next**.

    ⇒ The **Start Copying Files** window appears **(Figure C.6)**.

    This is a summary of the information selected.

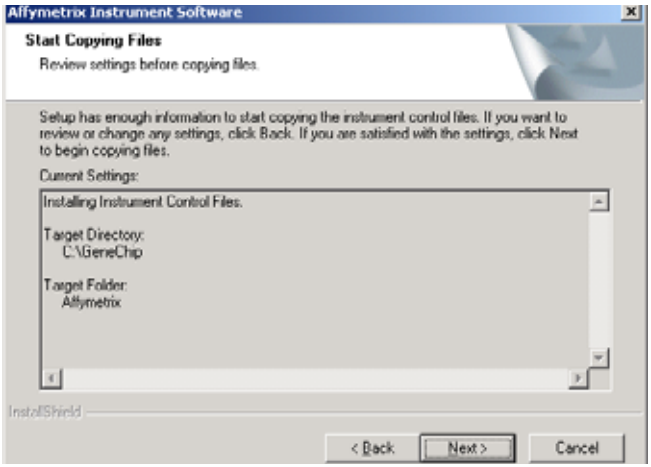


Figure C.6
Start Copying Files Window

13. Review the information and click **Next** to continue.

    ⇒ Program files and device drivers are copied to your system, and the Setup Complete window appears **(Figure C.7)**.

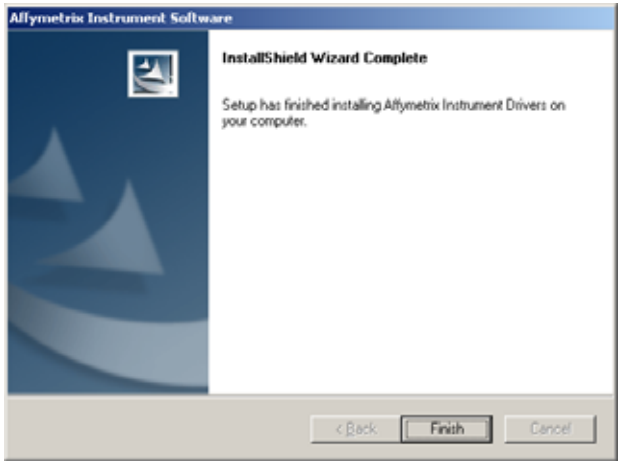


Figure C.7
Install Complete window

The Affymetrix® Scanner 3000 with Fluidics Station Installation

Follow this section if you have the GeneChip® Scanner 3000.

When you install the fluidics station software, confirm the presence of the correct Sealevel card.

✔ NOTE *If you cannot confirm the presence of a Sealevel card, contact Affymetrix technical support.*

1. On an instrument workstation that will be controlling fluidics stations, there must be a Sealevel card for communication between the workstation and the fluidics stations. Review **Figure C.8** to confirm that a Sealevel card is installed in your system.

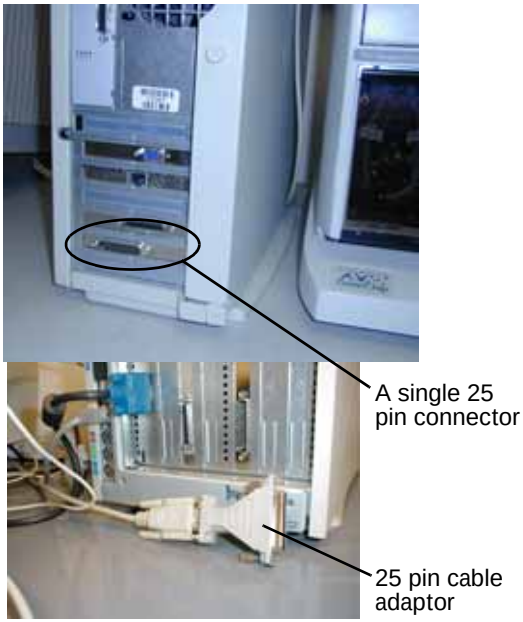


Figure C.8
Confirming the Sealevel card setup: note the 25 pin connector and the 25 pin cable adaptor

2. Launch **Windows 2000 Explorer**.
3. Browse to the Instrument CD.
4. Double-click **setup.exe** within the Instrument CD.
   ⇒ The **Welcome** window appears.
5. Click **Next**.
6. Several consecutive **Software License Agreement** windows appear. Click **Yes** in each window to accept the terms of the agreement.
   ⇒ The Choose Destination Location window appears **(Figure C.9)**.

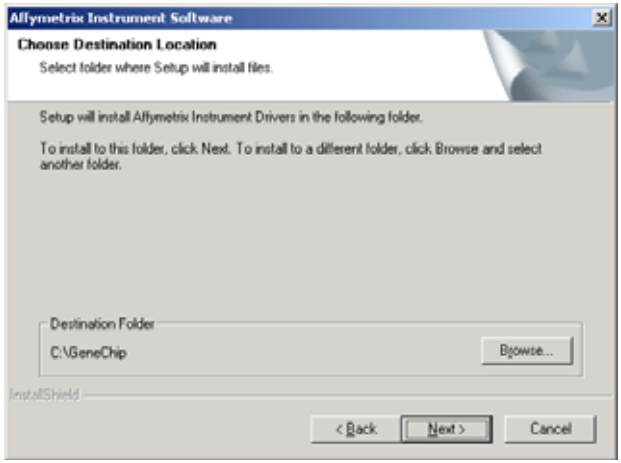


Figure C.9
Choose Destination Location window

7. Click Browse and select the **Destination** to install the instrument driver (select the **same location** where you installed GCOS).

8. Click **Next**.

   ⇒ The Select Components window appears **(Figure C.10)**.

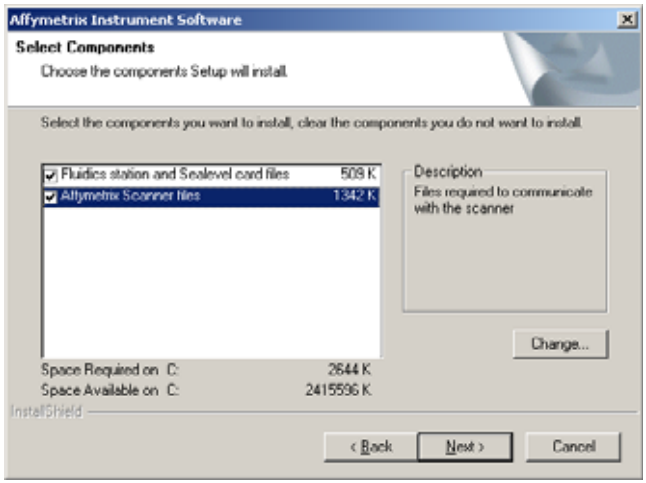


Figure C.10
Select both the Scanner Files and Fluidics Station and Sealevel card files

9. Select both the **Affymetrix Scanner Files** option and the **Fluidics Station Files** option **(Figure C.10)**. Highlight the **Affymetrix Scanner Files** option and click **Change...** to select the type of scanner.

   ⇒ The following dialog box opens and displays the choice of scanners, Affymetrix or GeneArray® **(Figure C.11)**.

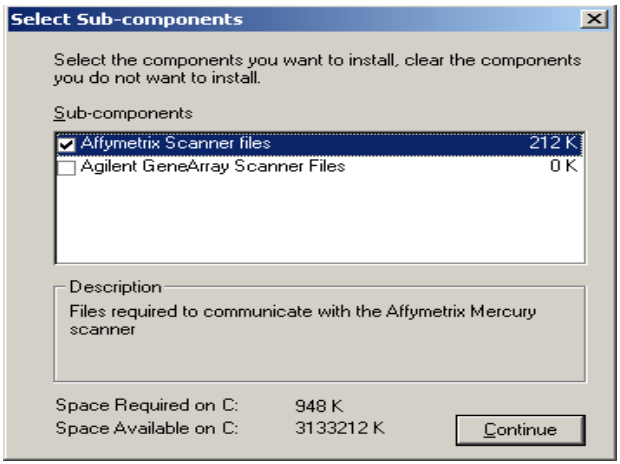
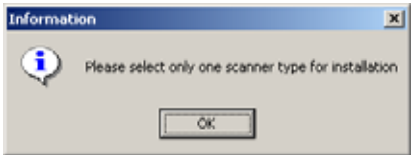


Figure C.11
Select the Affymetrix scanner. Select one scanner but not both.

10. Select the Affymetrix Scanner files and click **Continue**.

    If you click **Continue** with both scanners selected, the software will display the following message.



11. Click **Next** and enter the Port # for the COM serial port for the Sealevel serial card. Enter **2** **(Figure C.12)**.

✔ NOTE

*If the workstation is the **Dell GX110**, select **COM Port 3**.*

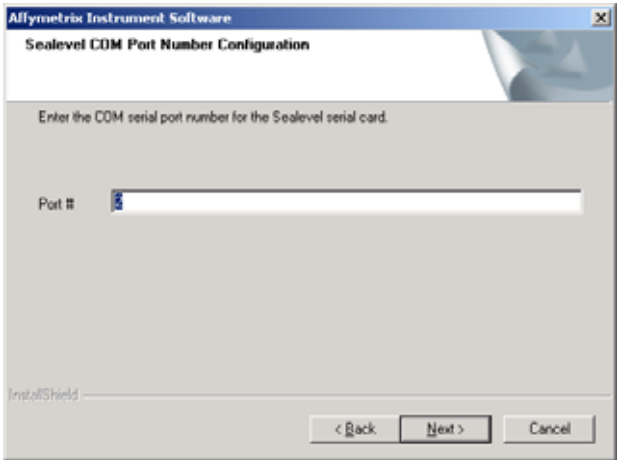


Figure C.12
Select the Port Number for the Sealevel card.

12. Click **Next**.

    ⇒ The **Start Copying Files** window appears **(Figure C.13)**.

    This is a summary of the information selected.

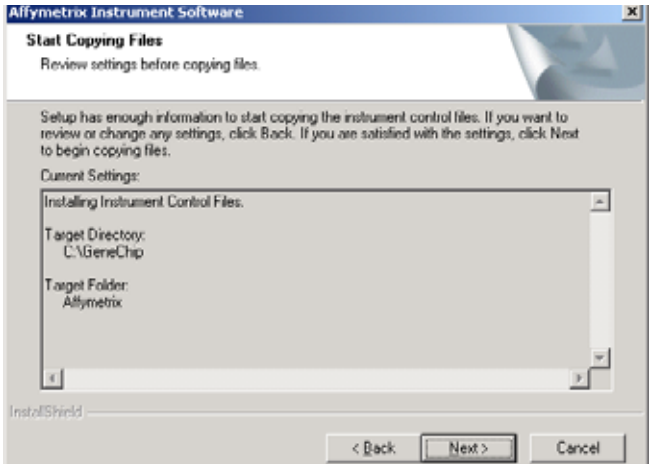


Figure C.13
Start Copying Files Window

13. Review the information and click **Next** to continue.
    ⇒ Program files and device drivers are copied to your system, and the Setup Complete window then appears **(Figure C.14)**.

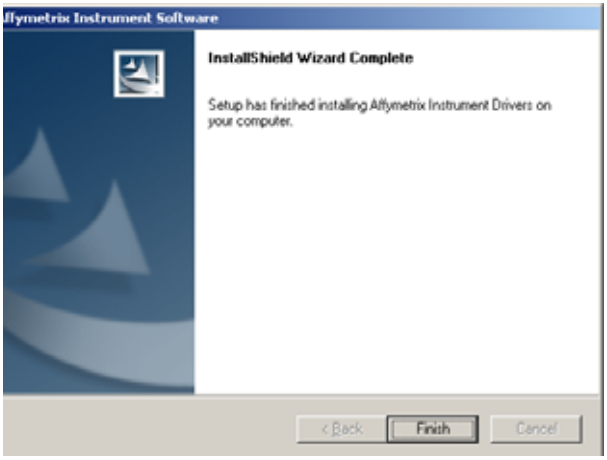


Figure C.14
Install Complete window

14. Click **Finish**.

Fluidics Station Installation (only)

When you install the fluidics station software, confirm the presence of the correct Sealevel card.

✔ NOTE *If you cannot confirm the presence of a Sealevel card, contact Affymetrix technical support.*

1. On an instrument workstation that will be controlling fluidics stations, there must be a Sealevel card for communication between the

workstation and the fluidics stations. Review **Figure C.15** to confirm that a Sealevel card is installed in your system.

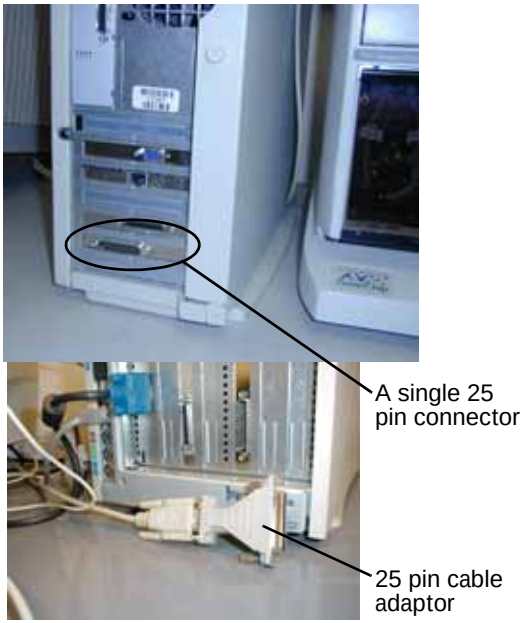


Figure C.15
Confirming the Sealevel card setup: note the 25 pin connector and the 25 pin cable adaptor

2. Launch **Windows 2000 Explorer**.
3. Browse to the Instrument CD.
4. Double-click **setup.exe** within the Instrument CD.

   ⇒ The **Welcome** window appears.
5. Click **Next**.
6. Several consecutive **Software License Agreement** windows appear. Click **Yes** in each window to accept the terms of the agreement.

   ⇒ The Choose Destination Location window appears **(Figure C.16)**.

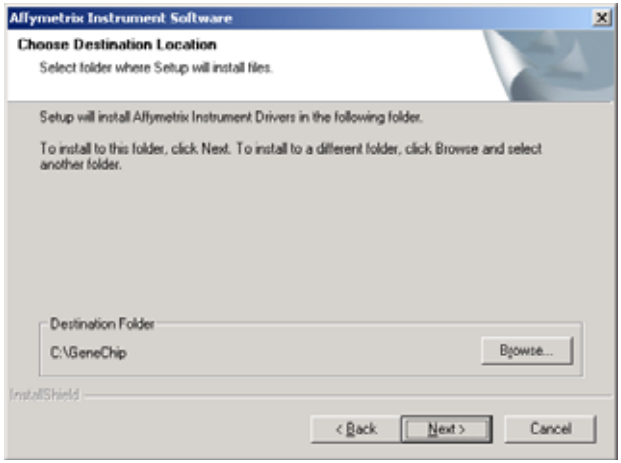


Figure C.16
Choose Destination Location window

7. Click Browse and select the **Destination** to install the instrument driver (select the **same location** where you installed Microarray Suite).

8. Click **Next**.

   ⇒ The Select Components window appears **(Figure C.17)**.

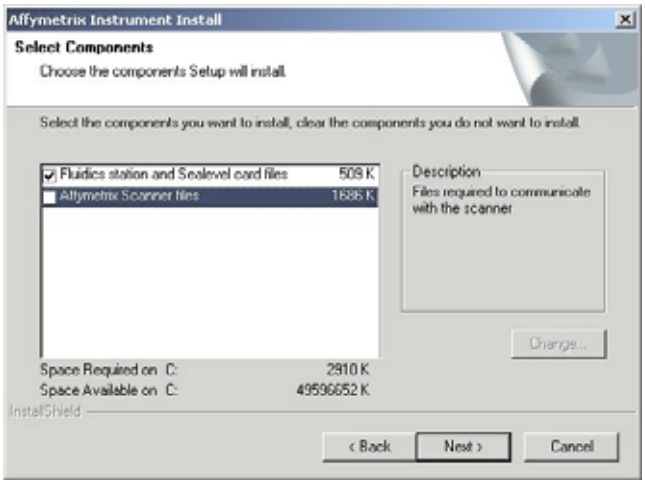


Figure C.17
Select only the Fluidic Station and Sealevel card files.

9. Select the **Fluidics Station Files** option **(Figure C.17)**.
   This should be the only option selected.
10. Click **Next**.
11. Enter the Port # for the COM serial port for the Sealevel serial card. Enter **2** **(Figure C.18)**.

✔ NOTE *If the workstation is the GX110, GX260 or GX270, select COM Port 3.*

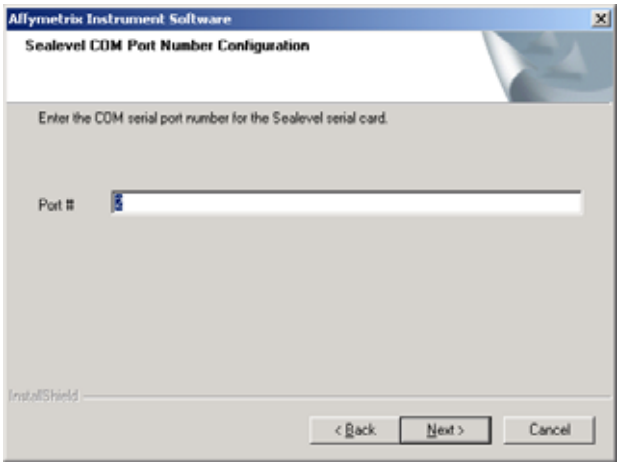


Figure C.18
Select the Port Number (Sealevel card only)

12. Click **Next**.
    ⇒ The **Start Copying Files** window appears **(Figure C.19)**.
    This is a summary of the information that you selected.

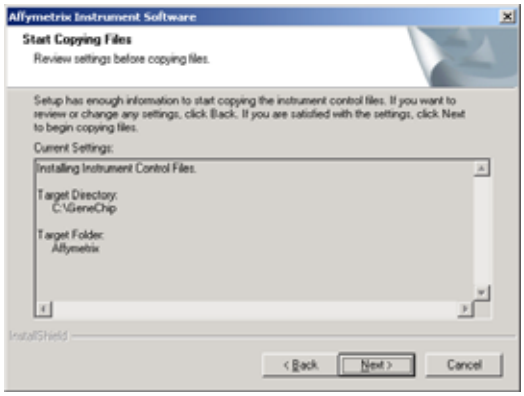


Figure C.19
Start Copying Files Window

13. Review the information and click **Next** to continue.

    ⇒ Program files and device drivers are copied to your system, and the Install Complete window appears **(Figure C.20)**.

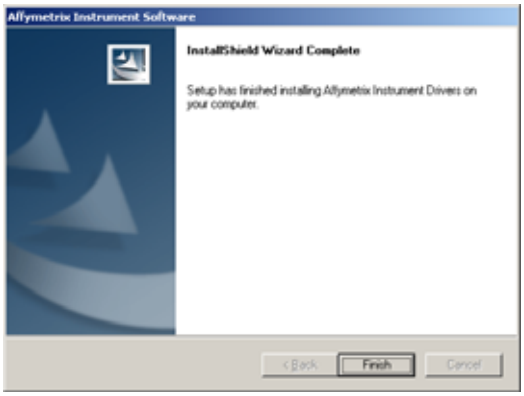


Figure C.20
Install Complete window

14. Click **Finish**.

GeneArray® 2500 Scanner Installation (only)

1. Launch **Windows 2000 Explorer**.
2. Browse to the Instrument CD.
3. Double-click **setup.exe** within the Instrument CD.

   ⇒ The **Welcome** window appears.
4. Click **Next**.
5. Several consecutive **Software License Agreement** windows appear. Click **Yes** in each window to accept the terms of the agreement.

   ⇒ The Choose Destination Location window appears **(Figure C.21)**.

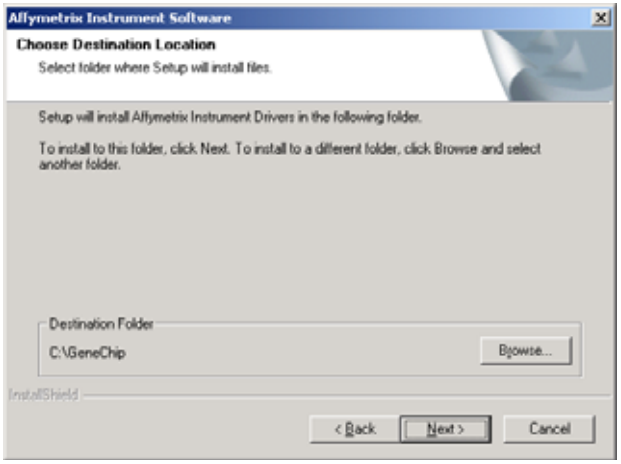


Figure C.21
Choose Destination Location window

6. Click Browse and select the **Destination** to install the instrument driver (select the **same location** where you installed Microarray Suite).
7. Click **Next**.

   ⇒ The Select Components window appears **(Figure C.22)**.

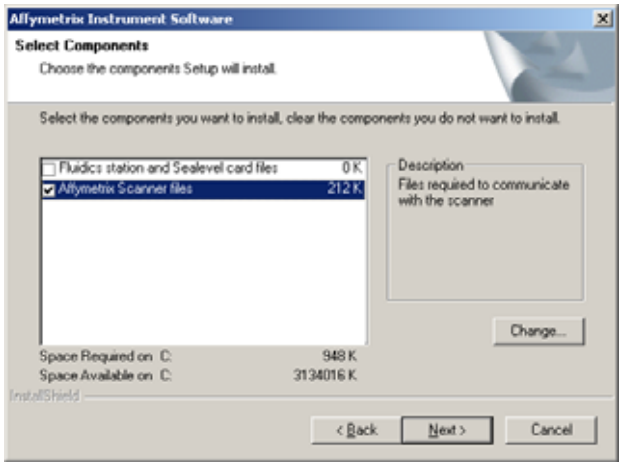


Figure C.22
Select only Scanner files for scanner installation

8. Select only the **Affymetrix Scanner Files** option **(Figure C.22)**. Highlight the **Affymetrix Scanner Files** option and click **Change...** to select the type of scanner.

   ⇒ The following dialog box opens and displays the choice of scanners, Affymetrix or GeneArray® **(Figure C.23)**.

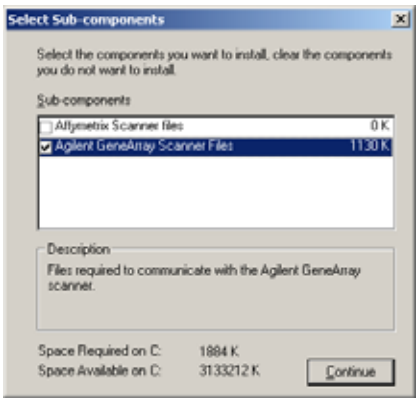
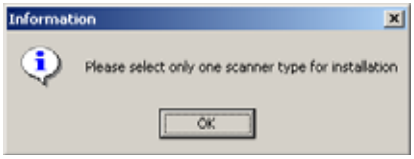


Figure C.23
Select the GeneArray® scanner. Select one scanner but not both.

9. Select the GeneArray® 2500 scanner Files and click **Continue**.

   If you click **Continue** with both scanners selected, the software will display the following message.



10. Click **Next**.

    ⇒ The **Start Copying Files** window appears **(Figure C.24)**.

    This is a summary of the information that you selected.

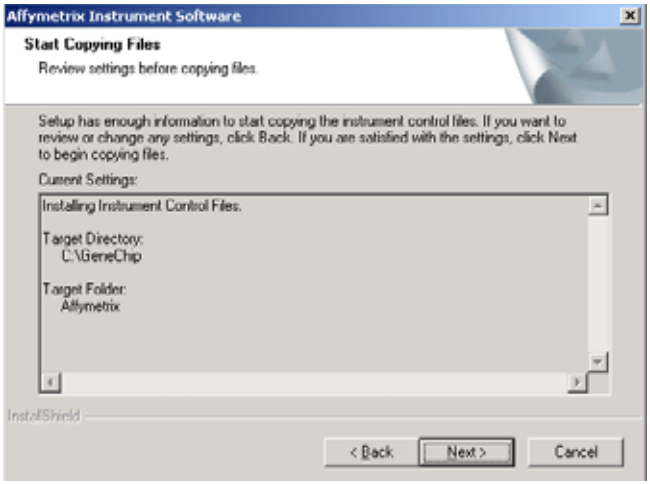


Figure C.24
Start Copying Files Window

11. Review the information and click **Next** to continue.

    ⇒ Program files and device drivers are copied to your system.

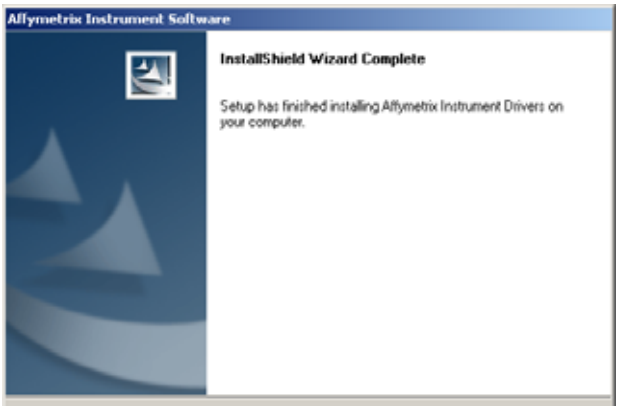


Figure C.25
Install Complete window

12. Click **Finish**.

GeneChip® Scanner 3000 Installation (only)

✔ NOTE

The instrument installation is for Microsoft® Windows 2000 Workstation only.

1. Launch **Windows 2000 Explorer**.
2. Browse to the Instrument CD.
3. Double-click **setup.exe** within the Instrument CD.
   ⇒ The **Welcome** window appears.
4. Click **Next**.
5. Several consecutive **Software License Agreement** windows appear. Click **Yes** in each window to accept the terms of the agreement.
   ⇒ The Choose Destination Location window appears **(Figure C.26)**.

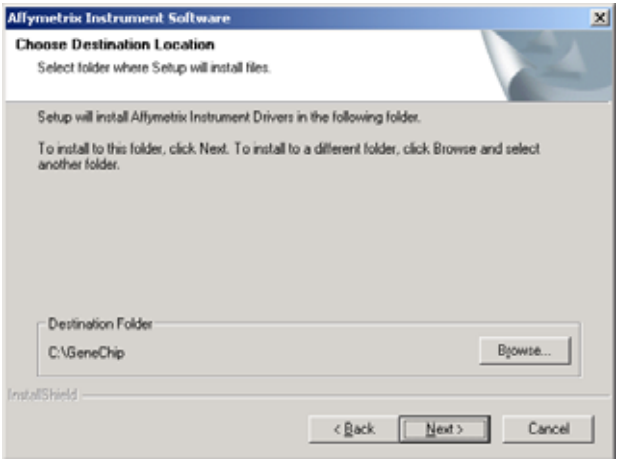


Figure C.26
Choose Destination Location window

6. Click Browse and select the **Destination** to install the instrument driver (select the **same location** where you installed Microarray Suite).
7. Click **Next**.
   ⇒ The Select Components window appears **(Figure C.27)**.

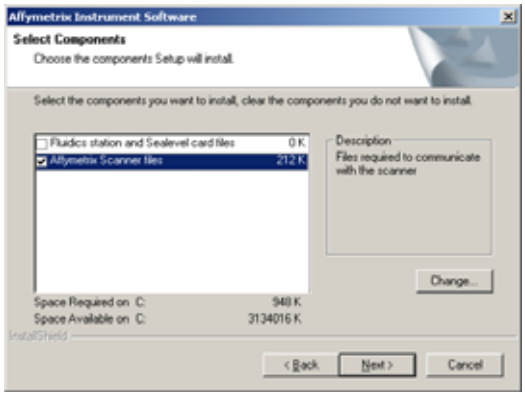


Figure C.27
Select Affymetrix Scanner files only for scanner installation

8. Select only the **Affymetrix Scanner Files** option. Highlight the **Affymetrix Scanner Files** option and click **Change...** to select the type of scanner.

   ⇒ The following dialog box opens and displays the choice of scanners, Affymetrix or GeneArray® **(Figure C.28)**.

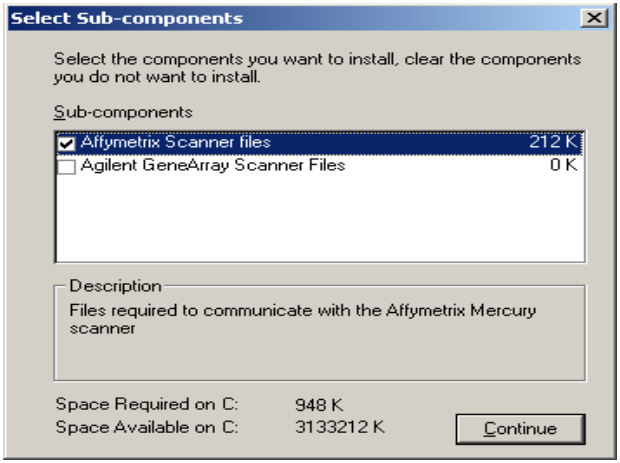
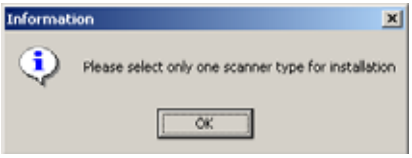


Figure C.28
Select the Affymetrix scanner. Select one scanner but not both.

9. Select the Affymetrix Scanner files and click **Continue**.

   If you click **Continue** with both scanners selected, the software will display the following message.



10. Click **Next**.

    ⇒ The **Start Copying Files** window appears **(Figure C.29)**.

    This is a summary of the information that you selected.

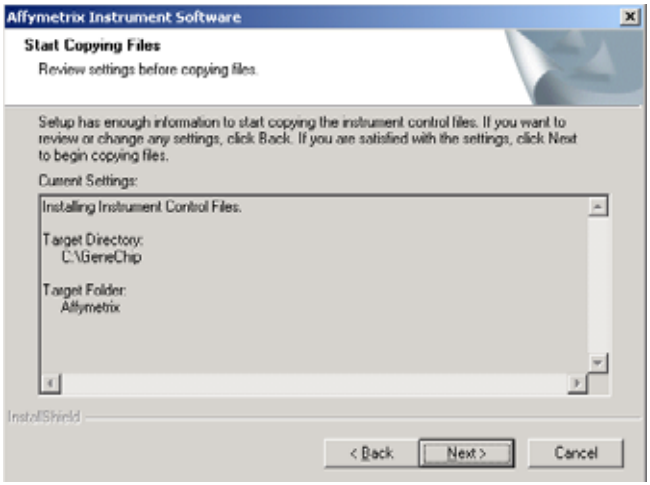


Figure C.29
Start Copying Files Window

11. Review the information and click **Next** to continue.

    ⇒ The Install Complete window appears **(Figure C.30)**.

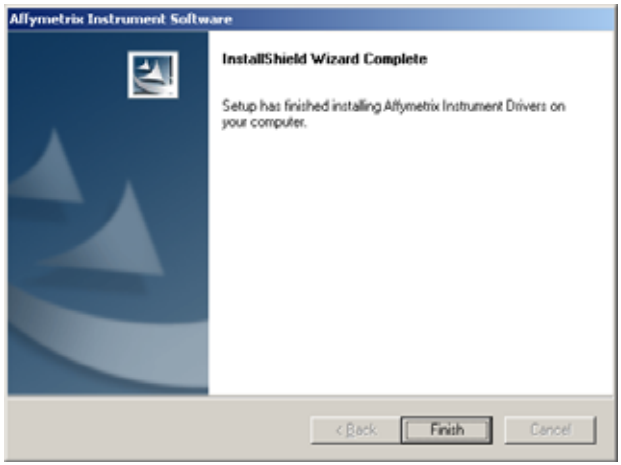
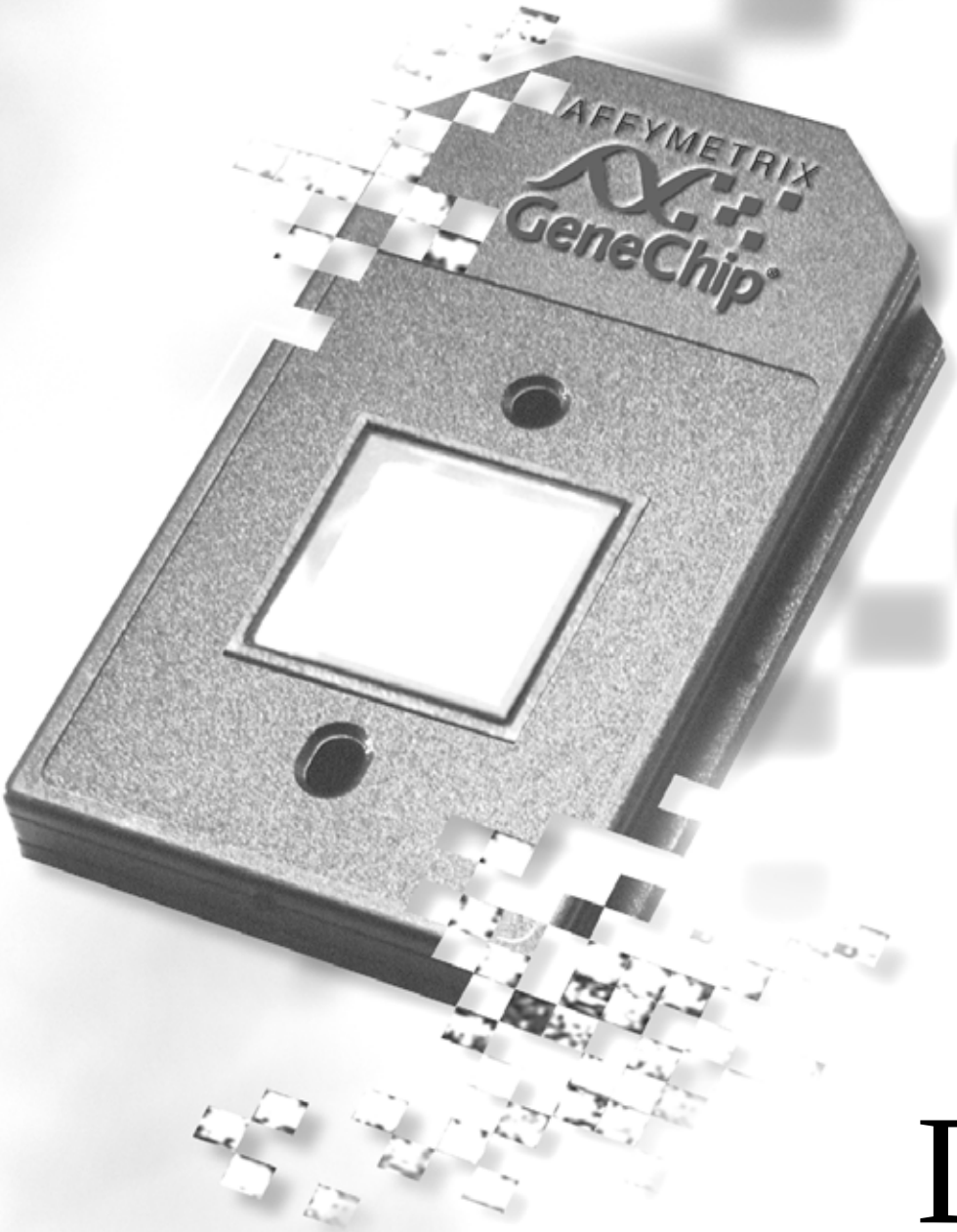


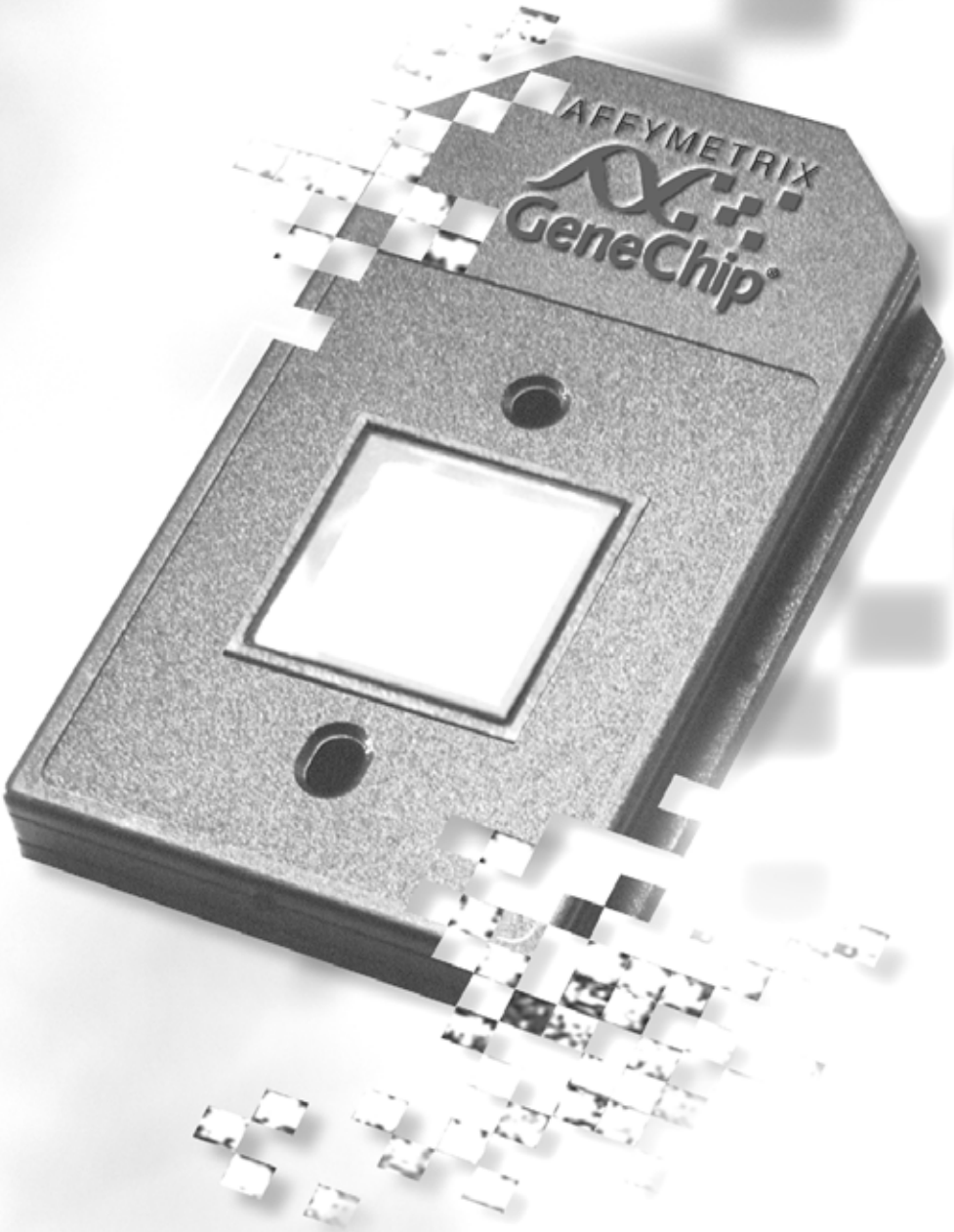
Figure C.30
Install Complete window

12. Click **Finish**.



D

Appendix D



Using the GeneChip® Scanner 3000

Affymetrix has designed a new scanner expressly for scanning GeneChip® probe arrays. The GeneChip® Scanner 3000 requires little maintenance.

The instrument must be kept clean and free of dust. Dust buildup can degrade performance. Wipe the exterior surfaces clean using a mild dish detergent solution in water. Do not use ammonia based cleaners or organic solvents, such as alcohol or acetone, to clean the system because they may damage the exterior surfaces.

Figure D.1
The Affymetrix® GeneChip® Scanner 3000

! CAUTION *Do not remove the cover of the scanner. Use the scanner only as instructed in this User Guide. Do not attempt to service the instrument.*

Introduction

The Affymetrix® GeneChip® Scanner 3000 is a wide-field, epifluorescent, near-confocal microscope. The scanner uses a 532 nm solid-state laser to excite probe array fluorophores. This in turn produces an emission wavelength at about 570 nm. As the surface of the probe array is scanned, a photomultiplier tube collects and converts the fluorescent emissions into an electrical signal. An analog-digital converter in the scanner converts this signal into corresponding numeric values representative of fluorescent intensities. These digital intensity values are collected from discrete areas on the array surface and are stored on the computer workstation as pixels that comprise the image data file (the .dat file). See Chapter 9, *Working with Images*, on page 135 for more on image data.

Affymetrix' patented "flying objective™" technology represents a radical departure from conventional laser scanners. The optical system comprises a scan arm that rapidly oscillates from side to side scanning the entire width of the probe array in a continuous arc while the probe array is advanced in front of the objective. The acquired image of the array is returned to the computer software as a set of arcs. The software then geometrically corrects these arcs to form a linear image of the array **(Figure D.2)**.

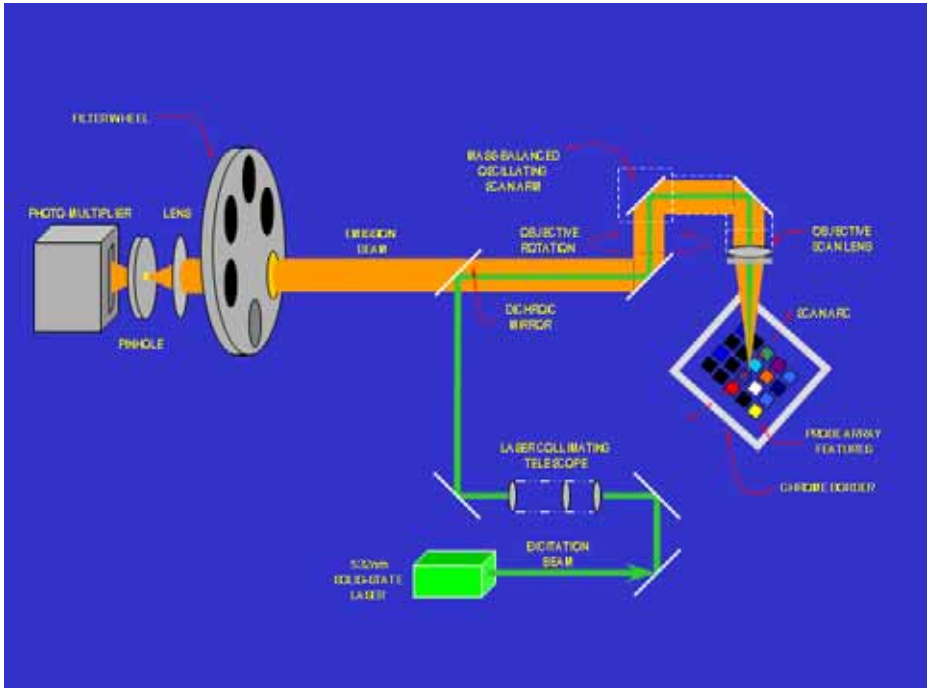


Figure D.2
Schematic of the scanner design

The laser source excites the hybridized fluorophores and the photomultiplier system simultaneously captures the resulting fluorescent intensities. The optical components direct the fluorescent beam back through the objective lens, through a dichroic mirror and to the PMT. An analog-digital converter transforms the PMT output into 65536 levels of intensity. Each level of intensity is stored in the software as a 16 bit number (2^{16}=65536).

The scanner is equipped with an IEC 320 compliant power entry module located at the rear of scanner **(Figure D.3)**.

The scanner is equipped with an RJ-45 interface connector compatible with 10/100 Base T Ethernet for communications with the host workstation.

The GCOS application controls the scanner. The scan process times for each type of GeneChip® probe array are outlined in the next table. Affymetrix measures the process time from the "Start Scan" command to the return of

the cartridge to the instrument entrance port along with the display of the completed scan image on the workstation monitor.

Probe Array Type	Process Time (typical)
49	5min 15sec
64	4min 30sec
100	3min 30sec
169	3min 30sec
400	2min 45sec

After the scanner has completed a scan, GCOS displays a picture of the image in the image window. The software displays the fluorescent intensity values from each pixel within the probe array feature in a grayscale or pseudocolor mode and superimposes a grid on the image to delineate the probe cells.

GCOS analyzes the image and derives a single intensity value for each probe cell on an array. This data is automatically generated and saved to the cell intensity file. See Chapter 9, *Working with Images,* on page 135.

Safe Operation

To ensure safe operation of the GeneChip® Scanner 3000:

- Read this section and *Scanning a Probe Array–GeneChip® Scanner 3000,* on page 111 completely before operating the instrument.
- Do not attempt to service this instrument. Any attempt at unauthorized service may damage the instrument and/or void the warranty.
- The instrument weight is approximately 63 pounds (28.6 Kg). Do not place it on an unstable cart, stand, or table. Failure to properly support the instrument may cause serious damage or injury and may void the warranty.

! CAUTION *Heavy object. Two people are required to lift the scanner.*

- The instrument must be surrounded by adequate airspace. Slots and openings in the instrument and the electronics compartment covers are for ventilation. Do not block or cover them.

- Never push an object into the instrument ventilation slots; equipment damage or injury may result. Do not set liquids on top of the instrument.
- The instrument has an AC receptacle with a safety ground appropriate for the country of destination. The plug is designed to connect only to a 3-prong ground receptacle. This safety feature should not be compromised in any way. If the instrument AC plug does not mate with the available power source receptacle, consult a licensed electrician to install one that does.

When to Contact Affymetrix

Under any of the following conditions, unplug the instrument from the power source and contact technical Support:

- When the power cord is damaged or frayed.
- If any liquid has been spilled into the instrument.
- If the instrument has been penetrated by water.
- If, after service or calibration, the instrument does not perform in accordance with the capabilities stated in the specifications.
- If the instrument has been dropped or otherwise damaged.

If the instrument must be returned for repair, call Affymetrix® Technical Support.

WARNING *Do not remove the cover of the scanner. Use the scanner only as instructed in this User Guide. Do not attempt to service the instrument. Only qualified service technicians can open and service the scanner. There are no customer serviceable parts. Removing the case exposes the customer to laser and electrical shock hazards.*

Laser Safety

The laser is equipped with an automatic shutter that inhibits its output beam and ensures safe operation under conditions encountered in normal operation. The instrument covers, probe array cartridge access port, and protective shutters ensure that during instrument operation no directed or stray laser light leaves the instrument.

IMPORTANT *The GeneChip scanner 3000 is a Class I laser product when the laser is enclosed in scanner case. The laser itself is a Class IIIB laser product.*

DANGER

Laser radiation when open.
Avoid direct exposure to beam.

The lasers can cause serious injury if the instrument is not operated in accordance with instructions in this user guide.

CAUTION

Use laser safety glasses when servicing
DO NOT STARE INTO BEAM.

Class I Laser Product

The green laser is a 532nm solid-state laser. This is a Class IIIb laser and has visible outputs greater than 5mw but no more than 500mw. It must never be operated in an exposed manner. Any object in the direct path of the laser beam may be damaged. Eyes and skin can be seriously damaged by direct exposure to, specular reflections from, or diffuse reflections from this laser. If improperly used, a laser of this type can cause fires. When used according to the instructions in this manual and when all covers are in place, the GeneChip® Scanner 3000 is classified as a Class I Laser Device per 21 CFR 1040.

Always take note of laser safety labels; they indicate areas where exposure to laser beams may be hazardous.

Electrical Safety

The scanner will automatically handle any input voltage from 100 to 240 VAC nominal, 50 to 60 Hz

✔ NOTE *The scanner's power supply will autodetect the input voltage source and configure itself.*

CAUTION

CAUTION

The power supply cord is used as the main disconnect device. Ensure that the socket outlet is located and installed near the equipment and is easily accessible.

ATTENTION

Le cordon d'alimentation est utilisé comme interrupteur general. La prise de courant doit être située ou installée a proximité du materiel et être facile d'accés.

ACHTUNG

Zur sicheren Trennung des Gerätes vom Netz ist der Netzstecker zu ziehen. Vergewissern Sie sich, daß die Steckdose leicht zugänglich ist.

Connections and Indicator Lights

The GeneChip® Scanner 3000 has the following connections on the back of the unit **(Figure D.3)**:

- AC power connection
- Ethernet connection

If you must move the scanner, disconnect all cables first.

Connecting the Scanner

1. Connect the 3-pronged electric power plug to the workstation.
2. Connect the dedicated Ethernet cable from the scanner to the Ethernet port that is designated on the workstation. The port is located near the bottom of the workstation.
3. You can connect your company's network cable to the indicated port near the center of the workstation.

When you start GCOS, the software will make the proper communication connections.

! CAUTION

Do not confuse your company's network connections with the dedicated Ethernet port of the scanner-workstation. The proper scanner connection is located near the bottom of the workstation.

This 10/100 Base T Ethernet communications port is dedicated to the scanner-workstation interface. You cannot connect the scanner to your company's Ethernet communications network.

You can, however, connect the workstation's second Ethernet port to the your company's Ethernet network.

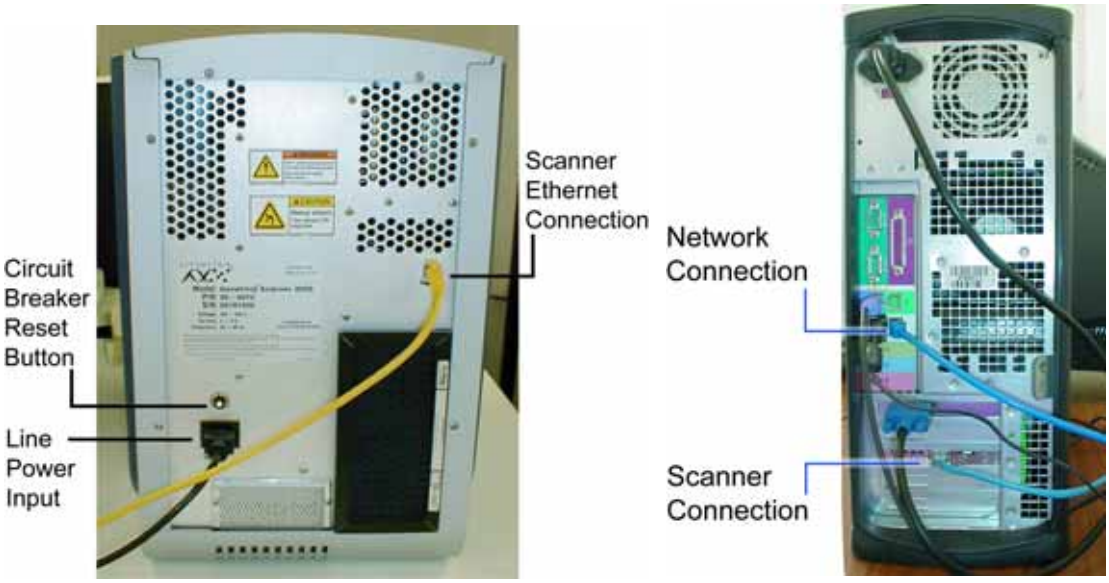


Figure D.3
Scanner Connections- left: rear of scanner -right: rear of computer

✔ NOTE

The reset button is the scanner's circuit breaker. The breaker switch will be tripped whenever the scanner experiences an electrical fault condition. Press to reset. If you cannot reset this switch, contact Affymetrix technical support.

Indicator Lights and On/Off Button

The front panel has the following button and indicators **(Figure D.4)**.

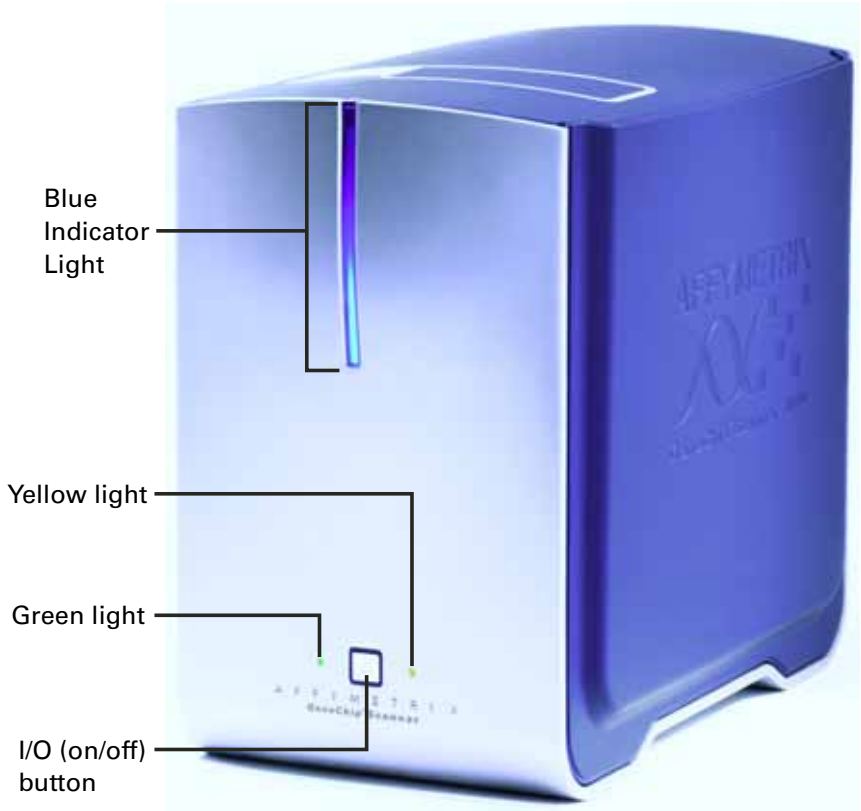


Figure D.4
Scanner indicator lightsn and on/off (I/O) button

1. I/O (on/off) button in the center.
2. Blue indicator light, running vertical at front center, indicates that the scanner is on.
3. Green and yellow light both on indicate scanner boot up in progress.

4. Yellow light
   a. On = Idle, laser is warming up (laser not ready, green off)
   b. Off = System ready, no errors (Green on)
   c. Flashing = Error
5. Green light
   a. On = System is ready to scan (yellow off)
   b. Flashing = Scan in progress

Summary of Indicator Lights

The table below summarizes the light conditions and their meaning.

Condition	Green Light	Yellow Light	Blue Indicator Light	Meaning
Initial boot up	Off	Off	On	Initial power up; embedded PC takes control
Scanner boot up	On	On	On	Embedded PC takes control of scanner boot up
Laser warm up	Off	On	On	Software enabled and laser is warming up
System ready	On	Off	On	User communication with scanner enabled
Error	Off	Flashing	On	Fatal error, reboot scanner and software
Scanning enabled	Flashing	Off	On	Scanning in progress

✔ NOTE

During the initial power up (CPU boot up), both the green and yellow lights will be off.

During the scanner boot up, both the green and yellow lights will be on.

Using the Barcode Reader

If your scanner is equipped with a barcode reader, follow this section for instructions on its use.

1. Open the GCOS software to the Experiment Information window and place the cursor in the barcode reference field of the GCOS Experiment Information window **(Figure D.5)**.

IMPORTANT

You should open a new experiment and place the cursor into the barcode field before triggering the reader. Otherwise, the barcode will be misinterpreted as some other kind of input. You are required to open a new experiment for each barcode and must enter certain other information before the experiment can be saved. See **Table L.3,** *on page 674 for a summary of information that you can enter in the Experiment Information Window.*

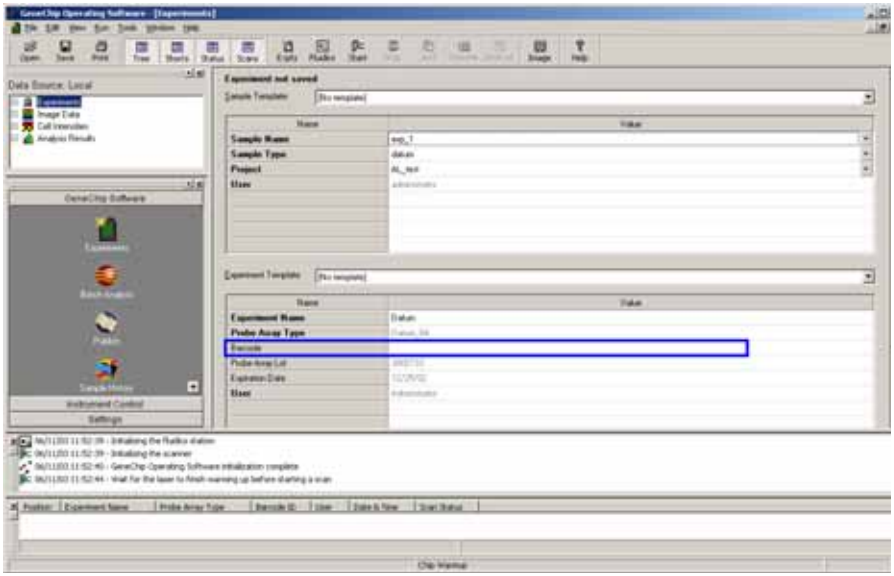


Figure D.5
Experiment Information Window with cursor in the barcode field

2. Hold a GeneChip probe array cartridge in front of the barcode reader and squeeze the trigger for approximately four seconds until you hear a beep **(Figure D.6)**.

Figure D.6
Reading the cartridge barcode

3. The reader reads and sends the barcode to the GCOS Experiment Information window, Barcode field **(Figure D.7)**.

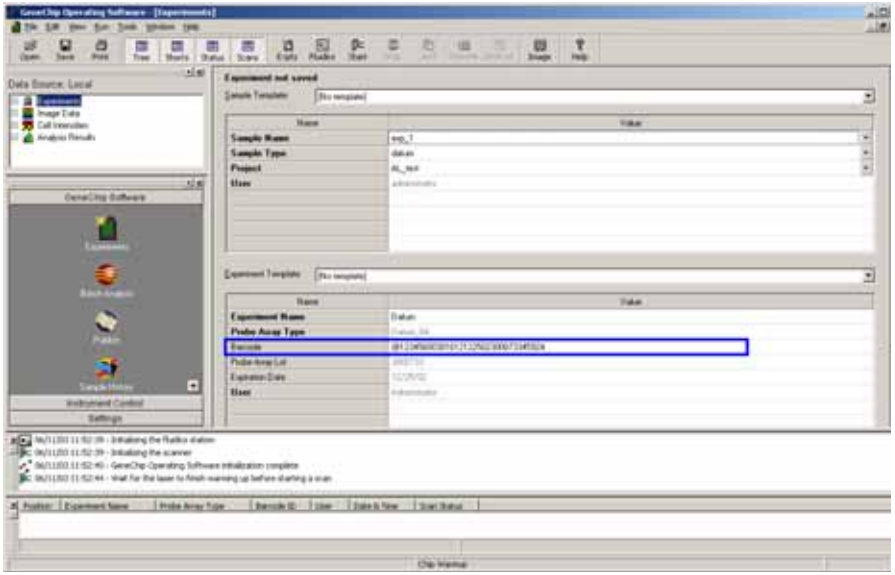


Figure D.7

4. After the software adds the barcode, save the experiment.
5. Repeat steps 1 to 4 until all of the probe array cartridges have been read.

Scanner 3000 Specifications

Item	Parameter	Value
Weight	Shipping	approx 78 pounds (35.4 Kg)
	Free-standing	63 pounds (28.6 Kg)
Dimensions	Width	~13.25 in.
	Depth	~27.5 in.
	Height	~18.25 in.

Item	Parameter	Value
Power	Voltage Current Line Frequency	100 - 240 V ~ 4 - 2 A 50 - 60 Hz
Working Environment	Temperature	59°F-85°F (15°C-30°C)
	Humidity	10-90% Non-condensing
	Clearance	2 in. (5 cm) on side, back and top
	Pollution Degree	2
	Installation Category	II
	Altitude	<2000m
Electrical Supply	Provide voltage, frequency or power rating per unit label	
Main Supply Voltage Fluctuations	Are not to exceed ±10% of the nominal supply voltage	

! CAUTION *If you use the scanner in a manner not specified in this user guide, you may impair the protection provided by the equipment.*

Troubleshooting

Problem	Possible Cause	Corrective Action
No image when scanning	Power off or cable loose	Check all connections and power.
	Loss of laser power	Contact technical support.
Intermittent problems scanning	Loose cable	Check all rear connections.
Scanner fails with probe array inside	Power failure	Manually extract probe array. Check all connections to scanner. Turn scanner on, restart software.

Issues Relating to the Scanner's Operation

The GeneChip® Scanner 3000 is a new type of scanner. In using the scanner you may encounter some issues, or problems, that may require your intervention. Below is a list of these issues.

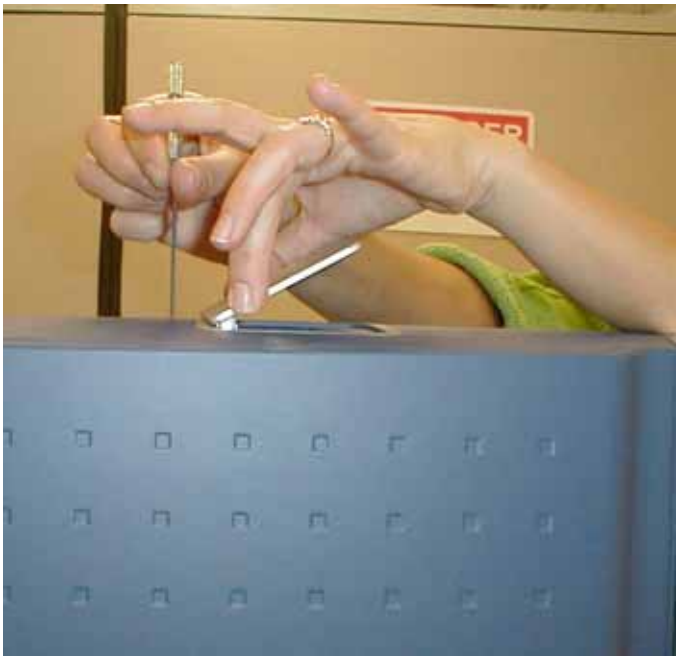
Issue	Explanation
If communications are interrupted during a scan (by a faulty cable connection or power being lost at the scanner, for example)	GCOS will properly note the failure and present a message "Cannot connect to Scanner." However, there are two issues to note. First, GCOS will report such a failure only after a network time-out of about 30 seconds. Second, rarely, if communications have been lost, GCOS and the Scanner may not be able to automatically restore communications once the problem is rectified, and both may become unresponsive. To restore proper operation, verify that the scanner is on, that communication cables are properly connected, and close and restart Windows then restart GCOS. If the system remains unresponsive, disconnect and reconnect power to the scanner, restart the scanner normally, close and restart Windows and GCOS.
Repeated attempts to send commands (Start, Turn Laser On, etc.) from GCOS to the Scanner while GCOS is reporting the scanner "Offline" may result in GCOS becoming unresponsive until communications are restored	If communications cannot be re-established, please follow the recommendations of item 1
If the Scanner experiences multiple auto-focus failures, the system may enter an unresponsive state.	Follow the recommendations of item 1 to restore communications and correct operation.
Laser warm-up lasts for ten minutes, during which time the "Turn Laser On" button will remain unchanged and GCOS will display the status message "Warm-up".	Simply note that this is normal operation.

Issue	Explanation
Occasionally, when observing DAT images in GCOS, the user may notice, at specific zoom levels, that grid lines do not properly appear.	Reducing the graphics acceleration parameter in Windows desktop Properties will eliminate this problem.
If no chip is inserted and a scan started.	The scanner will attempt go through the first parts of the auto-focus routine and then report “Failed to find chrome border”.
The scanner should be in the park mode to eject the chip.	
Auto focus will fail if salt deposits accumulate on the array.	Use Tough-Spots to prevent leaks in the GeneChip® probe array. See the quick reference card, p/n 08-0076.

Manually Removing a Lodged Probe Array Cartridge

In the event that a probe array becomes lodged in the chip transport mechanism, follow the procedure outlined below.

1. Turn off the scanner.
2. Insert a paper clip or small Allen wrench into the rescue hole on top of the scanner and press to partially lift the cartridge loading door.

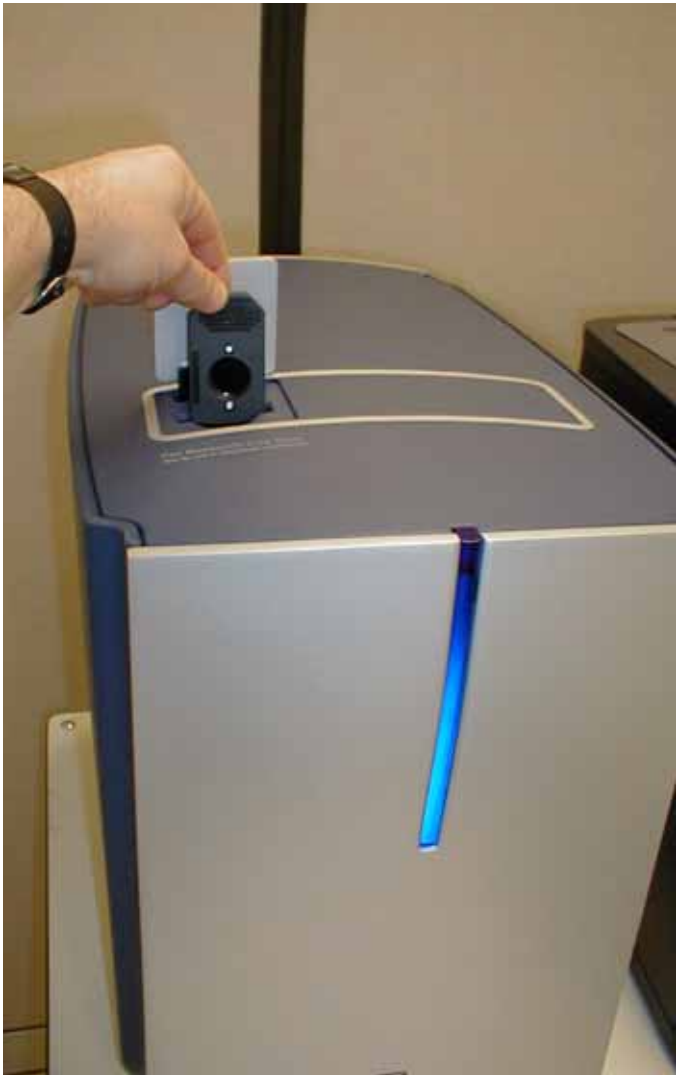


3. Using your fingers, gently lift the front edge of the door. As you lift the front edge, lift the back edge approximately 1/4" to open the door straight up to expose the rescue screw in the front.

4. Using a standard (-) screwdriver, turn the rescue screw clockwise to raise the chip transport mechanism.
5. Continue to turn the screw until the probe array cartridge ascends sufficiently to grab it.

Note that the screw is fine pitched and requires a number of turns. Stop if you encounter screw resistance. Do not over torque.

6. When the cartridge has ascended sufficiently, remove it.
7. Rescrew the chip transport mechanism until it descends completely, or until you encounter resistance. Do not over torque.
8. Close the door.



CE Mark Declaration of Conformity

We, Affymetrix, Inc.
4G Crosby Drive,
Bedford, Massachusetts

Declare under sole responsibility that the **Affymetrix® GeneChip® Scanner 3000** conforms with the relevant provisions of the following standards or other normative documents:

EU EMC Directive 89/336/EEC:

EN 61326:1998	Equipment for Measurement, Control and Laboratory Use, EMC Requirements
EN 55011:1998	Industrial, scientific and medical (ISM) radio-frequency equipment - Radio disturbance characteristics - Limits and methods of measurement
EN 61000-3-2:2001	Limits for harmonic current emissions (equipment input current up to and including 16 A per phase)
EN 61000-3-3:1995	Limitation of voltage changes, voltage fluctuations and flicker in public low-voltage supply systems, for equipment with rated current less than or equal to 16 A per phase and not subject to conditional connection
EN 61000-4-2:1995	Electrostatic discharge immunity.
EN 61000-4-3:1995	Radiated, radio frequency, electromagnetic field immunity.
EN 61000-4-4:1988	Electrical fast transient/burst immunity.
EN 61000-4-5:1995	Surge immunity.
EN 61000-4-6:1996	Immunity to conducted disturbances induced by radio frequency fields.
EN 61000-4-11:1994	Voltage dips, short interruptions, and voltage variations immunity.

EU Low Voltage Directive 73/23/EEC

EN 61010-1:2001	Safety requirements for electrical equipment for measurement, control, and laboratory use -- Part 1: General requirements
EN 60825-1:1994+ A2:2001	Safety of laser products -- Part 1: Equipment classification, requirements and user's guide

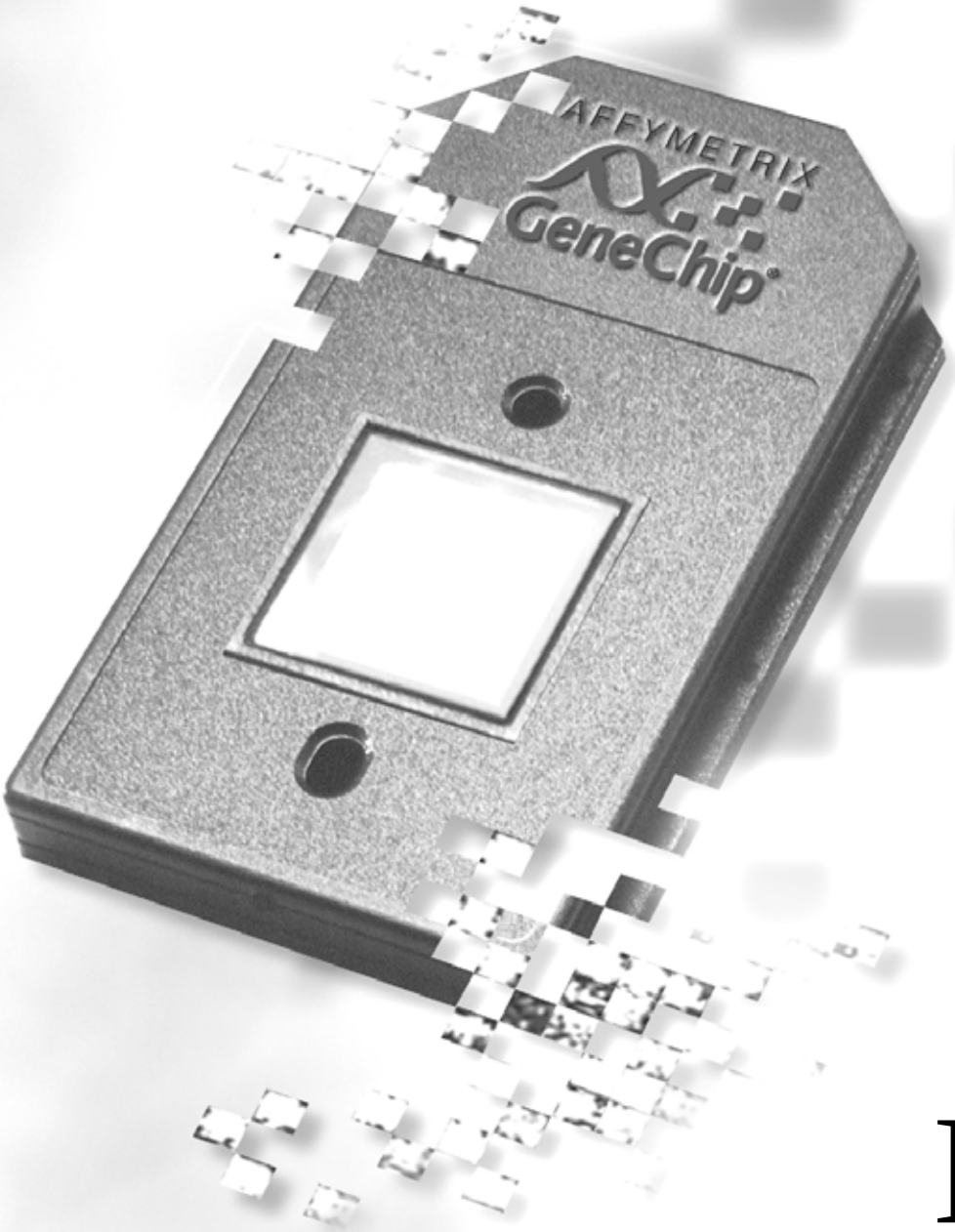
Regulatory

This device complies with **Part 15, Subpart B, Class B of the FCC rules**. *Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesirable operation.*

This device complies with FDA performance standards for laser products except for deviations pursuant to Laser Notice No. 50, dated July 26, 2001.

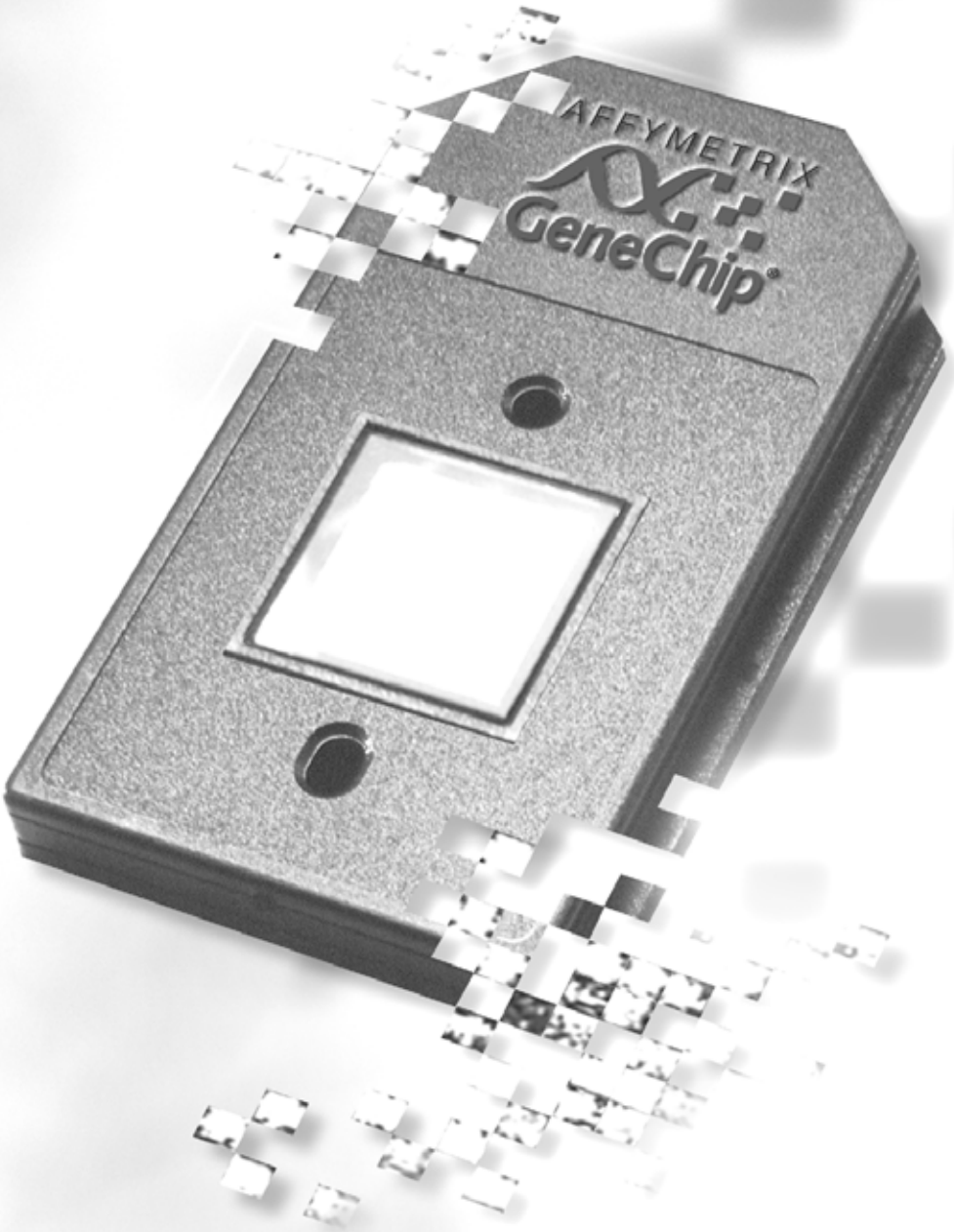
Regulatory Agency	Certification
TÜV PRODUCT SERVICE GS	AL 02 12 39816 004
c UL us LISTED	92AA
CE	See the section entitled *CE Mark Declaration of Conformity* on page 510.
Class I Laser Device	21 CFR 1040.10 and 1040.11

For Research Use Only. Not for use in diagnostic procedures.



E

Appendix E



Expression Algorithm, Expression Analysis Metrics & Settings

GCOS and versions of Microarray Suite higher than 5.0 *run the Statistical expression algorithm. Previous versions of Microarray Suite (lower than 5.0) run the Empirical expression algorithm.*

Expression Analysis Algorithm

Notation

A GeneChip® probe array consists of a number of *cells* (square-shaped areas on the array) and each contains many copies of a unique probe. Probes are tiled in probe pairs consisting of a perfect match (PM) and a mismatch (MM).

The sequence of the PM and MM are the same, except for a base substitution in the middle of the MM probe sequence. A probe set includes a series of probe pairs and represents an expressed transcript **(Figure E.1)**.

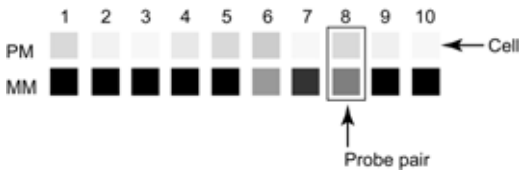


Figure E.1
Probe set that includes 10 probe pairs

Algorithm Output Definitions

Signal	A measure of the abundance of a transcript.
Stat Pairs	The number of probe pairs for a particular probe set on the array.
Stat Pairs Used	= Pairs - Masked probe pairs - Saturated MM probe pairs This is the number of pairs used by the Statistical Expression algorithm to make the detection call in an single-array analysis.
Detection	The call in an single-array analysis that indicates if the transcript was Present (P), Absent (A), Marginal (M), or No call (NC).
Detection *p*-value	*p*-value that indicates the significance level of the Detection call.
Stat Common Pairs	The intersection of the probe pairs from the baseline and experiment that are used by the Statistical Expression algorithm to make the Change call in a comparison analysis.
Change	The call that indicates the change in transcript level between a baseline and an experiment array.
Change *p*-value	*p*-value that indicates the significance level of the Change call.
Signal Log Ratio	The change in expression level for a transcript between a baseline and an experiment array. This change is expressed as the $\log_2$ ratio.
Signal Log Ratio low	The lower limit of the Log Ratio within a 95% confidence range.
Signal Log Ratio high	The upper limit of the Signal Log Ratio within a 95% confidence range.

Background Subtraction

The first step in the analysis is to correct for background across the entire array. The calculated background establishes an intensity *floor* that is subtracted from all intensity values.

Raw cell intensities →Background-adjusted intensities

The algorithm:

- divides the array into equally spaced zones **(Figure E.2)**
- assigns an average background to the center of each zone
- computes the distance from each cell to the center of every zone
- computes a weighting factor (the reciprocal of the sum of a constant and the square of the distance from the cell to the zone center)
- computes the background of each cell by applying the weighting factor to the zone average (the average background assigned to the center of each zone)

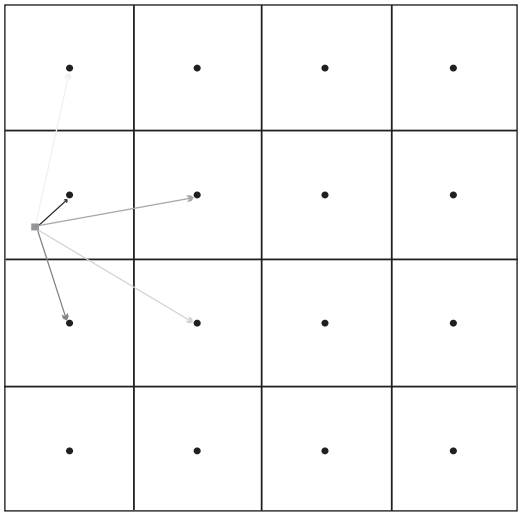


Figure E.2
Array zones for computing background; the arrow color indicates the relative weights

Single-Array Expression Analysis

Detection

The Detection call answers the question: Is the transcript of a particular probe set reliably detected by the probe array? We want an answer of *Absent* or *Present*. In this context, Absent means the expression level is below the threshold of detection. In the case of uncertainty, we can get a *Marginal* call.

Detection call results are easy to filter and interpret. For example, we may only want to look at genes whose transcripts are Present in a particular experiment. An additional advantage is that the Detection and the Signal values are calculated using independent algorithms that add additional information to the results.

Raw cell intensities →Absent, Present, or Marginal Detection call plus *p*-values

✔ NOTE

A No Call detection result occurs if all of the probe pairs of a probe set are excluded from the analysis. A probe pair is excluded if the PM or MM is masked or if the MM is saturated.

Discrimination Value

The algorithm computes a discrimination value that is used as a filter to remove from further consideration all probe sets with insignificant differences between PM and MM.

discrimination value = (PM - MM)/(PM + MM)

The median of the discrimination ratios of all the probe pairs of a probe set is compared to a user-modifiable parameter τ (default = 0.015), and produces an intermediate call.

✔ NOTE

Increasing τ can reduce the number of false Present calls, but may also reduce the number of true Present calls.

Making the Call

A one-sided Wilcoxon's Signed Rank test is used to calculate a *p*-value that reflects the significance of the differences between PM and MM. The *p*-value or statistical significance of a result is the probability that the

observed change in a sample occurred by pure chance. For example, a *p*-value of 0.05 means there are five chances in 100 that the results are not significant. The lower the *p*-value, the greater the probability that the results are significant.

To make a call, the *p*-value for a probe set is examined on an axis with two user-definable thresholds, α_1 and α_2 (defaults $\alpha_1 = 0.04$ and $\alpha_2 = 0.06$). For *p*-values between zero and 0.5, α_1 and α_2 define the thresholds for the calls **(Figure E.3)**. See also **Table E.1**.

The result is reported as the *Detection call* that is associated with the calculated *p*-value.

✔ NOTE

Decreasing the significance level α_1 can reduce the number of false detected calls and reduce the number of true detected calls.
Increasing the significance level α_2 can reduce the number of false undetected calls and reduce the number of true undetected calls.

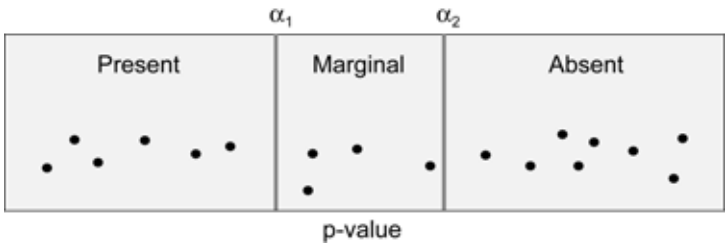


Figure E.3
User-modifiable thresholds, α_1 and α_2

Table E.1
Statistical algorithm Detection call rules

Computed Detection *p*-values	Detection Call
$p < \alpha_1$	Present
$\alpha_1 \leq p < \alpha_2$	Marginal (at the limit of detection)
$p \geq \alpha_2$	Absent

Calculating the Signal

The signal represents the amount of transcript in solution.

Background-adjusted cell intensities →Probe set signal

For each PM intensity, a matching MM probe provides a reference background hybridization intensity. If the MM value is less than the PM value, the algorithm uses the MM value directly. However, if the MM value is larger than the PM value, the algorithm creates an adjusted MM value based on the average difference intensity between $\log_2$ PM and $\log_2$ MM, or if that measure is too small, some fraction of PM.

The adjusted MM values are used to calculate the $\log_2$ PM - log adjusted MM for each probe pair. The Signal for a probe set is calculated as the one-step biweight estimate of the combined differences of all the probe pairs in the probe set.

Comparison Expression Analysis

A comparison analysis compares the expression levels of the transcripts on one array to those on another. By directly comparing matching cells on two arrays, any inherent differences in hybridization efficiency will cancel out. As a result, this is an accurate and sensitive method of determining changes in expression levels.

Differences between PM and MM and Differences between PM and Background (Experiment and Baseline) →Change call and *p*-value

The algorithm computes a primary normalization factor and two additional normalization factors that straddle the primary normalization factor. The

spread between the normalization factors is determined by the perturbation parameter (d).

To determine the *p*-values, a signed rank analysis is carried out on the PM and MM differences for each probe pair in a probe set from the two arrays in the comparison. The resulting *p*-values are used to make the change calls.

To make a call, the *p*-value for a probe set is examined on an axis with four thresholds **(Figure E.4)**. For *p*-values between zero and 1.0, γ_1 and γ_2 define the thresholds for the calls.

The result is reported as the *Change call* that is associated with the calculated *p*-value (see **Table E.2**). The output *p*-value is the *critical p*-value. The critical *p*-value is the *p*-value that determines the Change call.

The critical *p*-value, p, is defined by the following:

- $p = \max(p_0, p_1, p_2)$, if $p_0 < 0.5$, $p_1 < 0.5$ and $p_2 < 0.5$
- $p = \min(p_0, p_1, p_2)$, if $p_0 > 0.5$, $p_1 > 0.5$ and $p_2 > 0.5$
- $p = 0.5$, otherwise

Figure E.4
User-modifiable parameters, γ_1 and γ_2

Table E.2
Statistical algorithm Change call rules

Computed Change p-values	Change call
$p_0 < \gamma_1$ $p_1 < \gamma_1$ $p_2 < \gamma_1$	Increasing
$p_0 < \gamma_2$ $p_1 < \gamma_2$ $p_2 < \gamma_2$	Marginally increasing
Else	No change
$p_0 > 1-\gamma_2$ $p_1 > 1-\gamma_2$ $p_2 > 1-\gamma_2$	Marginally decreasing
$p_0 > 1-\gamma_1$ $p_1 > 1-\gamma_1$ $p_2 > 1-\gamma_1$	Decreasing

You may adjust γ_1 and γ_2 by specifying different values for: γ_{1L}, γ_{1H}, γ_{2L}, γ_{2H}. See *Expression Analysis Settings*, on page 532.

Decreasing γ_1 can reduce the number of false Increase and Decrease calls, but can also reduce the number of true Increase and Decrease calls. Increasing γ_2 can reduce the number of false No-Change calls, but can also reduce the number of true No-Change calls. Increasing the perturbation parameter (d) can increase the number of true No-Change calls, but can also increase the number of false No-Change calls.

✔ NOTE

A No Call occurs if all of the probe pairs of a probe set are excluded from the analysis. A probe pair is excluded if the PM or MM in the experiment or baseline is masked or saturated.

Signal Log Ratio

Probes inherently hybridize with slightly different affinities. Relative expression values compare identical probes in the baseline and experiment. As a result, probe specific effects are canceled out.

Adjusted cell intensities (Baseline and Experiment) →Signal Log Ratio, Signal Log Ratio low, and Signal Log Ratio high

The Signal Log Ratio calculation is an extension of the signal calculation. The discrimination of the $\log_2$ ratio is used to correct for outlier probes. The one-step biweight method is used to compute the average $\log_2$ ratio of the probe set. The upper and lower limits of the 95th confidence interval are reported as Signal Log Ratio high and Signal Log Ratio low.

References

1. Hoaglin, D.C., Mosteller, R., Tukey, J.W. 2000. *Understanding Robust and Exploratory Data Analysis*, John Wiley & Sons, New York.
2. Hollander, M., Wolfe, D.A. 1999. *Nonparametric Statistical Methods* (second edition), John Wiley & Sons, New York.
3. Liu, W.M., Mei, R., Bartell, D.M., Di, X., Webster, T.Q., Ryder, T. 2001. Rank-based algorithms for analysis of microarrays, *Proceedings SPIE*, 4266.
4. Wilcoxon, F. 1945. Individual comparisons by ranking methods. *Biometrics*.1:80-83.
5. Roderick, J.A., Little, D., Rubin, B. 1987. *Statistical Analysis With Missing Data*. John Wiley & Sons, New York.

Expression Analysis Metrics

GCOS and versions of Microarray Suite higher than 5.0 run the Statistical expression algorithm. Versions of Microarray Suite (lower than 5.0) run the Empirical expression algorithm.

Statistical Expression Algorithm

Single-Array Results

Signal

A measure of the abundance of a transcript.

Detection

The call that indicates whether the transcript is detected (P, Present), undetected (A, Absent), or at the limit of detection (M, Marginal).

Detection p-value

p-value that indicates the significance of the Detection call.

Stat Pairs

The number of probe pairs for a particular probe set on an array.

Stat Pairs Used

The number of probe pairs per probe set used in the analysis. This may be the total number of probes per probe set on the probe array or the number of probe pairs in a pre-designated subset (for example, probe pairs specified by a probe mask file and/or a masked image). Stat Pairs Used = total probe pairs per probe set – (probe pairs masked in a mask file) – (probe pairs masked in the image) – (saturated MM probe pairs).

Comparison Results

Signal Log Ratio

The change in the expression level of a transcript between a baseline and an experiment array. This change is expressed as the $\log_2$ ratio. A $\log_2$ ratio of 1 is equal to a fold change of 2.

Signal Log Ratio Low

The lower limit of the Log Ratio within a 95% confidence interval.

Signal Log Ratio High

The upper limit of the Log Ratio within a 95% confidence interval.

Change

The call that indicates the change in the transcript level between a baseline and experiment (Increase (I), Marginal Increase (MI), No Change (NC), Marginal Decrease (MD), Decrease (D))

Change p-value

p-value that indicates the significance of the Change call.

Stat Common Pairs

The intersection of the probe pairs from the baseline and experiment that are used by the Expression algorithm to make the Change call in a comparison analysis.

Empirical Expression Algorithm

Versions of Microarray Suite lower than 5.0 run the Empirical Expression algorithm.

Single-Array Analysis Results

Positive & Negative

The number of probe pairs scored positive or negative. A probe pair is called positive if the intensity of the PM probe cell is significantly greater than that of the corresponding MM probe cell. A probe pair is called negative if the intensity of the MM probe cell is significantly greater than that of the corresponding PM probe cell. To evaluate the intensity, the algorithm calculates the ratio and difference associated with each probe pair and compares these values to the Statistical Difference Threshold (SDT) and the Statistical Ratio Threshold (SRT).

A probe pair is Positive if: PM – MM $\geq$ SDT *and* PM/MM $\geq$ SRT

A probe pair is Negative if: MM – PM $\geq$ SDT *and* MM/PM $\geq$ SRT

The SDT is a function of the noise (Q) and is calculated by the software: $SDT = Q * SDT_{mult}$. The SDT_{mult} and the SRT are user-modifiable parameters. The SDT_{mult} is set at 2.0 for the standard staining protocol or 4.0 for the antibody amplification protocol. (Refer to the *Expression Analysis Technical Manual* or the *HuSNP® Mapping Assay User Manual*). The default SRT value is 1.5.

✔ NOTE *Increasing the SDT_{mult} and SRT increases analysis stringency, reducing these thresholds decreases analysis stringency.*

The number of positive and negative probe pairs is determined for every probe set and are used to derive parameters that describe probe set performance.

Pairs

The number of probe pairs for a particular probe set on an array.

Pair Used

The number of probe pairs per probe set used in the analysis. This may be the total number of probes per probe set on the probe array or the number of probe pairs in a pre-designated subset (for example, probe pairs specified by a probe mask file and/or a masked image).

Pairs Used = total probe pairs per probe set – (probe pairs masked in a mask file) – (probe pairs masked in the image).

Pairs in Avg

A *trimmed* probe set that excludes probes with extremely intense or weak signal from the analysis. If 8 or fewer probe pairs are used, Pairs in Avg = Pairs Used (or the number of probe pairs per probe set minus any that are masked).

Super scoring is performed if more than 8 probe pairs are used. Superscoring is a process that excludes probe pairs from calculation of the Avg Diff and Log Avg Ratio if they are outside a given intensity range. Microarray Suite calculates the mean and standard deviation of the intensity differences (PM – MM) for an entire probe set (excluding the highest and lowest values). Those values within a set number of standard deviations (STP) are included in the calculation of the Avg Diff or Log Avg Ratio. The STP is a user-modifiable parameter with a default value = 3.

Pos Fraction

positive probe pairs/# probe pairs used

Log Avg

Describes the hybridization performance of a probe set and is determined by calculating the ratio of the PM/MM intensities for each probe pair in a probe set, taking the logs of the resulting values, and averaging them for the probe set:

Log Avg = 10 x {[Σ log (PM/MM)] / Pairs in Avg}

✔ NOTE

Log Avg = 0 indicates random cross hybridization. The higher the Log Avg, the more confidence the transcript is present.

Pos/Neg

The ratio of Positive probe pairs to Negative probe pairs in a probe set (# Positive probe pairs / # Negative probe pairs).

Avg Diff

$$\text{Avg Diff} = \frac{\sum(\text{PM} - \text{MM})}{\text{Pairs in Avg}}$$

This parameter serves as a relative indicator of the level of expression of a transcript. It is used to determine the change in the hybridization intensity of a given probe set between two different experiments.

The Avg Diff is calculated by taking the difference between the PM and MM of every probe pair (excluding the probe pairs where PM – MM is outside the STP standard deviation of the mean of PM-MM) in a probe set and averaging the differences for the entire probe set.

✔ NOTE *The Avg Diff cannot be used to compare the hybridization intensity levels of two different probe sets on the same array.*

Absolute Call

Each transcript in an single-array analysis has three possible Absolute Call outcomes: *Present* (P), *Absent* (A), or *Marginal* (M). The Absolute call is derived from the Pos/Neg, Positive Fraction, and Log Avg Absolute call metrics. Each Absolute call metric is weighted and entered into a decision matrix to determine the status of the transcript.

Comparison Analysis Results

Increase

A probe pair is considered to increase if the intensity difference between the PM and MM probe cells in the experimental sample is significantly higher than in the baseline sample. Two criteria must be met for a probe pair to show a significant increase:

$(\text{PM} - \text{MM})_{exp} - (\text{PM} - \text{MM})_{base} \geq$ Change Threshold (CT), *and*

$[(\text{PM} - \text{MM})_{exp} - (\text{PM} - \text{MM})_{base}] / \max [Q/2, \min(|\text{PM} - \text{MM}|_{exp} |\text{PM} - \text{MM}|_{base})] \geq$ Percent Change Threshold/100

Decrease

A probe pair is considered to decrease if the intensity difference between the PM and MM probe cells in the experimental sample is significantly lower than in the baseline sample. Two criteria must be met for a probe pair to show a significant decrease:

$(PM - MM)_{base} - (PM - MM)_{exp} \geq$ Change Threshold (CT), *and*

$[(PM - MM)_{base} - (PM - MM)_{exp}] / \max [Q/2, \min(|PM - MM|_{exp}, |PM - MM|_{base})] \geq$ Percent Change Threshold/100

The software calculates the Change Threshold (CT) using the SDT (Statistical Difference Threshold) of both the experimental and baseline data. Alternatively, the user may define the CT by entering a value for the CT Multiplier (in the Parameters tab of the Expression Analysis Settings dialog box), which is multiplied by the noise (Q) of the baseline or experimental data, whichever is greater. The Percent Change Threshold is a user-specified value (also set in the Parameters tab of the Expression Analysis Settings dialog box).

Inc Ratio

For each transcript: # Increased probed pairs / # probe Pairs Used

Dec Ratio

For each transcript: # Decreased Probe pairs / # probe Pairs Used

Pos Change

Positive probe $pairs_{exp}$ - # Positive probe $pairs_{baseline}$

Neg Change

Negative probe $pairs_{exp}$ - # Negative probe $pairs_{baseline}$

Inc/Dec

For each transcript: the # increased probe pairs / # decreased probe pairs

DPos-DNeg Ratio

(Positive Change – Negative Change)/# probe Pairs Used

The DPos – DNeg Ratio and Log Avg Ratio Change are usually positive when a transcript changes from a very low to a relatively high expression level and are typically negative when the expression level changes from a high to a very low or undetectable level. Both metrics may have values close to zero if the transcript is present in both the baseline and experimental samples despite an increase or decrease in the level of the transcript.

Log Avg Ratio Change

$\text{Log Avg}_{exp} - \text{Log Avg}_{base}$

The difference between the Log Avg Ratio of the baseline and experimental probe array data (in a comparison analysis) for each transcript. The Log Avg Ratios are recomputed for each for each probe set based on probe pairs used in both the baseline and experimental probe arrays (the recomputed values are not displayed by the software).

Difference Call

Each transcript in a comparison analysis has five possible Difference Call outcomes: (1) *Increase* (I), (2) *Marginally Increase* (MI), or (3) *Decrease* (D), (4) *Marginally Decrease* (MD), and (5) *No Change* (NC). The difference call is derived from the comparison metrics: Max [Increase/Total, Decrease/Total], Increase/Decrease Ratio, Log Average Ratio Change, and Dpos – Dneg Ratio. Each comparison metric is weighted and entered into a decision matrix to determine the status of the transcript.

Avg Diff Change

The Avg Diff values are recomputed for each probe set based on probe pairs used in both the baseline and experimental probe arrays (the recomputed values are not displayed by the software).

$\text{Avg Diff Change} = \text{Avg Diff}_{exp} - \text{Avg Diff}_{baseline}$

B=A

An asterisk (*) in this column indicates the transcript is called absent (A) in the baseline.

Fold Change

The Fold Change indicates the relative change in the expression levels between the experiment and baseline targets. The Fold Change for a transcript is a positive number when the expression level in the experiment increases compared to the baseline and is a negative number when the expression level in the experiment declines. The Fold Change (FC) is calculated as:

$$FC = \left(\frac{(AvgDiffChange)}{max[min(AvgDiff_{base}, AvgDiff_{\text{exp}}), Q_M \times Q_C]}\right) + \begin{Bmatrix} +1 \text{ if } (AvgDiff_{\text{exp}} \geq AvgDiff_{\text{base}}) \\ -1 \text{ if } (AvgDiff_{\text{exp}} < AvgDiff_{\text{base}}) \end{Bmatrix}$$

The normalized or scaled Avg Diff values are recomputed in both the experimental and baseline data sets to include only probe pairs used in both the baseline and experiment arrays. Then the Avg Diff Change is calculated as:

Avg Diff Change = Avg Diff_{exp} - Avg $\text{Diff}_{\text{base}}$

$Q_C = \max(Q_{\text{exp}}, Q_{\text{base}})$

Q_M = 2.1 for a 50µm feature or 2.8 for a 24µm feature

If the noise (Q) of the experiment or baseline array is greater than the Avg Diff of the transcript (the baseline or experimental data), the Fold Change is calculated over the noise and is an approximation [a tilde character (~) precedes the approximated Fold Change value in the *.chp file].

Sort Score

The Sort Score is a ranking based on the Fold Change and the Avg Diff Change. The higher the Fold Change and the Avg Diff Change, the higher the Sort Score.

Expression Analysis Settings

The expression analysis settings are user-modifiable variables with defaults empirically determined by Affymetrix. They are organized by the tabs of the Expression Analysis Settings dialog box:

- Scaling
- Normalization
- Probe Mask
- Baseline
- Parameters

The Expression algorithm relies on these settings to derive biologically meaningful results from the hybridization intensity data.

Viewing Expression Analysis Settings

1. Click **Analysis Settings** in the shortcut bar, then click **Expression**. Alternatively, select **Tools →Analysis Settings →Expression** from the menu bar.

   ⇒ The Expression Analysis Settings dialog box appears **(Figure E.5)**.

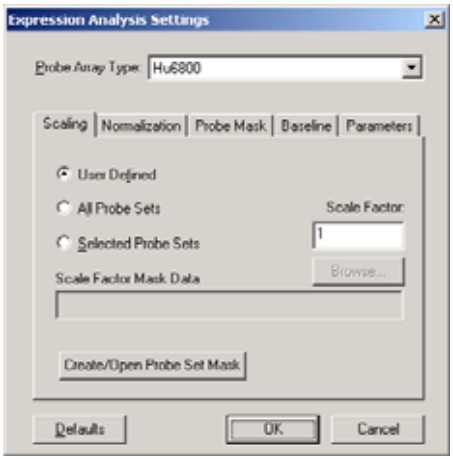


Figure E.5
Expression Analysis Settings, Scaling tab, User Defined scaling option selected

2. Make a selection from the **Probe Array Type** drop-down list.

   The settings are specific for the selected probe array type and do not affect the settings for other types of probe arrays.

3. Click a tab to view the different types of expression analysis settings for the selected probe array type.

Scaling Tab

Scaling is a mathematical technique applied to the data from several different probe arrays (of the same type) to minimize discrepancies due to variables such as sample preparation, hybridization conditions, staining, or probe array lot. The Scale Factor is also applied to the noise value.

GCOS offers three types of scaling: User Defined, All Probe Sets, and Selected Probe Sets.

To view the scaling settings:

- Click the Scaling tab in the Expression Analysis Settings dialog box **(Figure E.5)**.
- Make a selection from the **Probe Array Type** drop-down list.

User Defined Scaling

The **User Defined** scaling option multiplies the signal of each probe set on the array by a user-specified scale factor.

1. In the Scaling tab, choose the **User Defined** option **(Figure E.6)**.

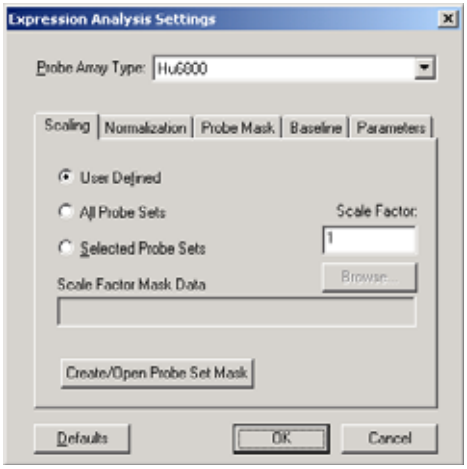


Figure E.6
Expression Analysis Settings, Scaling tab, User Defined scaling option with no scaling specified (scale factor = 1)

2. Enter a number in the **Scale Factor** box.

✔ NOTE *A scale factor of one is equivalent to no scaling.*

3. Click **OK** to close the Expression Analysis Settings dialog box.

All Probe Sets Scaling

The **All Probe Sets** scaling option adjusts the trimmed mean signal of a probe array to a user-specified target signal value.

The single-array analysis results (*.chp) from different experiments (probe arrays of the same type) that are scaled to the same target signal using the All Probe Sets scaling option may be directly compared.

1. In the Scaling tab, choose the **All Probe Sets** scaling option **(Figure E.7)**.

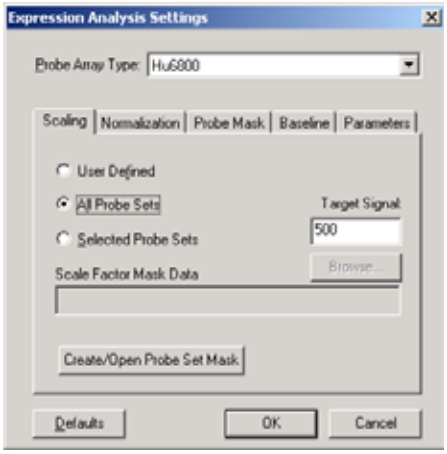


Figure E.7
Expression Analysis Settings, Scaling tab, All Probe Sets scaling option selected

2. Enter a target signal value.
3. Click **OK** to close the Expression Analysis Settings dialog box.

   GCOS examines all of the probe sets on the array to compute the trimmed mean signal and derive a scale factor for the array so that:

   Target Signal = Scale Factor x Trimmed Mean $\text{Signal}_{\text{probe array}}$

   The scale factor standardizes the trimmed mean signal of the array to the target signal **(Figure E.8)**.

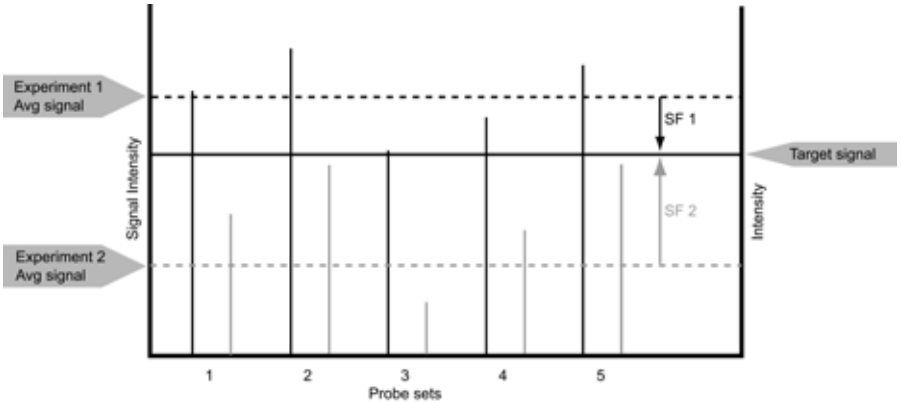


Figure E.8
Scale factors (SF) standardize the average signal of each experiment to a user-specified target signal

Selected Probe Sets Scaling

The **Selected Probe Sets** scaling option adjusts the trimmed mean signal of selected probe sets on a probe array to a user-specified target signal value.

You can directly compare the single-array analysis results (*.chp) of different experiments (probe arrays of the same type) if the same probe sets have been scaled to the same target signal in each experiment using the Selected Probe Sets scaling option.

For this scaling option, GCOS utilizes user-selected probe sets (specified by a Scale Factor mask file) to calculate the trimmed mean signal and derive the scale factor for the probe array so that:

$$\text{Target Signal} = \text{Scale Factor} \times \text{Trimmed Mean Signal}_{\text{selected probe sets}}$$

The scale factor standardizes the trimmed mean signal of an array to the target signal **(Figure E.8)**. Selected Probe Sets scaling does not change the single-array call because the software also multiplies the intensity of each probe set and the probe array noise by the scale factor.

Creating a Scale Factor Mask File

1. In the Scaling tab, choose the **Selected Probe Sets** option **(Figure E.9)**.

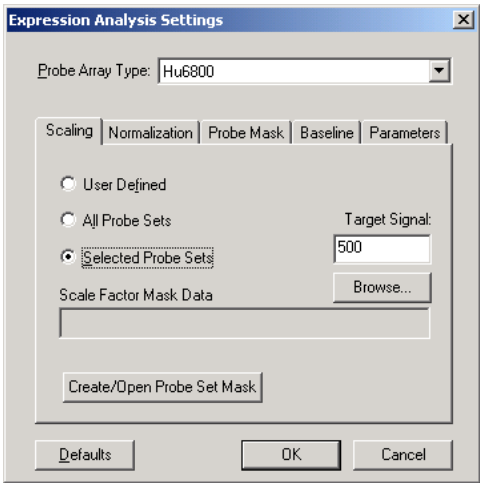


Figure E.9
Expression Analysis Settings, Scaling tab, Selected Probe Sets scaling option

2. Enter a **Target Signal** value, then click **Create/Open Probe Set Mask**.
   ⇒ The Probe Set Mask File dialog box displays existing mask files **(Figure E.10)**.

✔ NOTE

Mask files (.msk) include Scale Factor, Normalization, and Probe mask files. Each type of mask file has a different function and is created in a separate tab of the Expression Analysis Settings dialog box.*

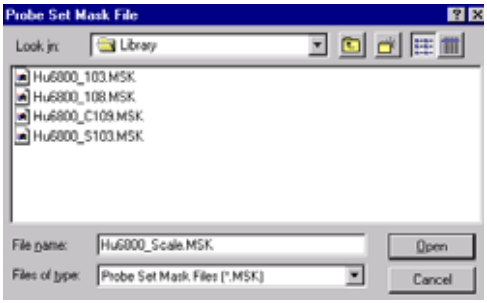


Figure E.10
Probe Set Mask Definition dialog box

3. Enter a name for the scale factor mask file in the **File name** box, then click **Open.**

   ⇒ The Probe Set Mask Definition dialog box appears **(Figure E.11)**.

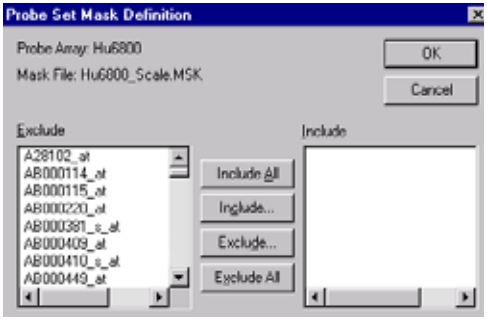


Figure E.11
Probe Set Mask Definition dialog box

4. To select probe sets for the mask file:

   a. Highlight the desired probe set names in the **Exclude** list.

   b. Click **Include**.

      ⇒ This adds the selected probe set names to the **Include** list for the scale factor mask file.

5. To remove a probe set name(s) from the **Include** list:

a. Highlight the name(s) in the **Include** list.

b. Click **Exclude**.

Include All automatically moves all of the probe set names from the **Exclude** list to the **Include** list.

Exclude All automatically Moves all of the probe set names from the **Include** list to the **Exclude** list.

6. Click **OK** when finished to create the scale factor mask file and close the Probe Set Mask Definition dialog box.

Selecting a Scale Factor Mask File

1. In the Scaling tab, choose the **Selected Probe Sets** option **(Figure E.12)**.

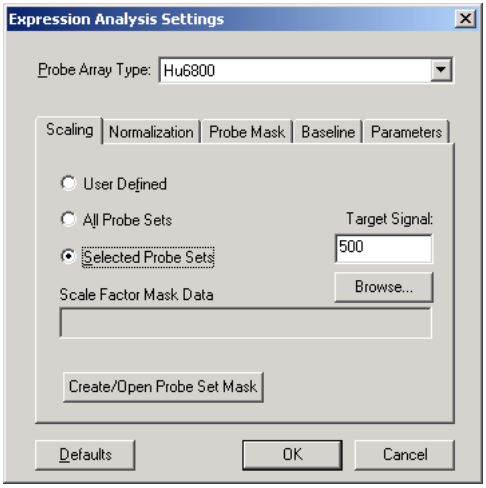


Figure E.12
Expression Analysis Settings, Scaling tab, Selected Probe Sets option

2. Click **Browse** **(Figure E.12)**.

   ⇒ The Probe Set Mask File dialog box appears **(Figure E.13)**.

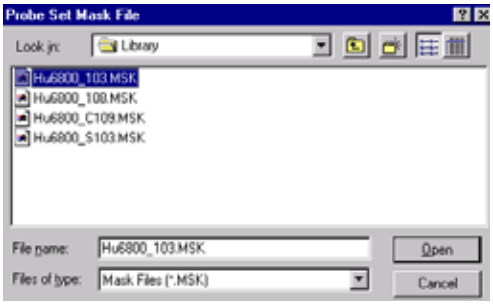


Figure E.13
Probe Set Mask File dialog box

✔ NOTE

Mask files (.msk) are specific for the probe array type. GCOS will not open a mask file that is incompatible with the currently selected probe array type.*

3. Double-click the desired *.msk file.

   ⇒ The Expression Analysis Settings dialog box displays the currently selected *.msk file name in the **Scale Factor Mask File** box **(Figure E.14)**.

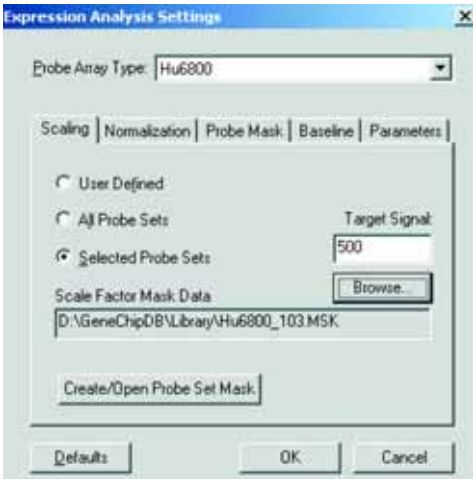


Figure E.14
Expression Analysis Settings, Scaling tab, scale factor mask file selected

Editing a Scale Factor Mask File

1. In the Scaling tab, choose the **Selected Probe Sets** option **(Figure E.15)**.

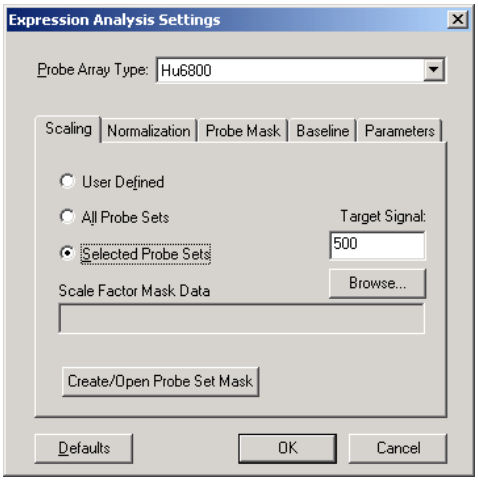


Figure E.15
Expression Analysis Settings, Scaling tab, Selected Probe Sets option

2. Click **Create/Open Probe Set Mask**.

   ⇒ The Probe Set Mask File dialog box appears **(Figure E.16)**.

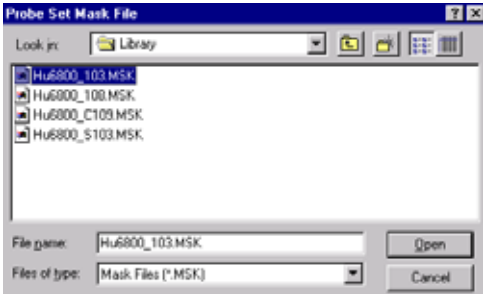


Figure E.16
Probe Set Mask File dialog box

3. Double-click the desired *.msk file.

   ⇒ The Probe Set Mask Definition dialog box appears **(Figure E.17)**.

   The **Include** list displays the probe sets included in the scale factor mask file. The **Exclude** list displays probe sets not included in the scale factor mask file.

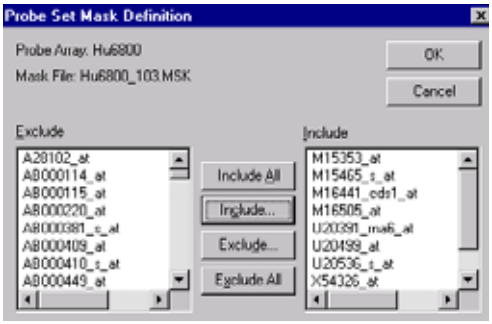


Figure E.17
Probe Set Mask Definition dialog box

4. To remove a probe set(s) from the *.msk file:

   a. Highlight the probe set name(s) in the **Include** list.

   b. Click **Exclude**.

5. To add a probe set(s) to the *.msk file, highlight the probe set name(s) in the **Exclude** list, then click **Include**.

   Include All automatically moves all of the probe set names from the **Exclude** list to the **Include** list.

   Exclude All automatically moves all of the probe set names from the **Include** list to the **Exclude** list.

6. Click **OK** when finished to close the Probe Set Mask Definition dialog box.

Normalization Tab

Normalization is a mathematical technique similar to scaling that enables comparison analysis of an experiment and baseline array.

GCOS offers three types of normalization: User Defined, All Probe Sets, or Selected Probe Sets normalization. All Probe Sets or Selected Probe Sets normalization minimizes discrepancies between an experiment and baseline array due to variables such as sample preparation, hybridization conditions, staining, or probe array lot.

User Defined Normalization

User Defined normalization multiplies the signal of each probe set on an array by a user-specified normalization value.

1. In the Normalization tab, choose the **User Defined** option **(Figure E.18)**.

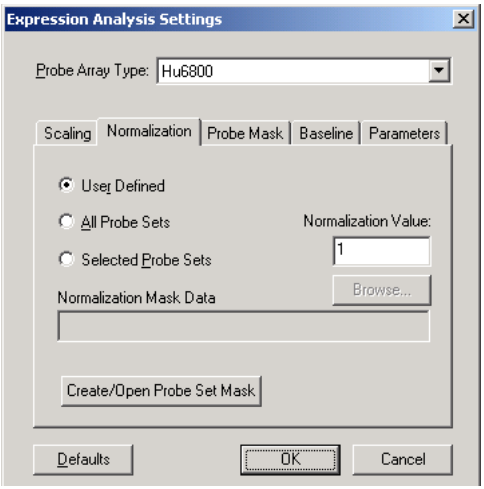


Figure E.18
Expression Analysis Settings, Normalization tab, User Defined normalization option with no normalization specified (Normalization Value = 1)

2. Enter a value in the **Normalization Value** box.

✔ NOTE *A normalization factor of one is equivalent to no normalization.*

3. Click **OK** to close the Expression Analysis Settings dialog box.

All Probe Sets Normalization

All Probe Sets normalization adjusts or *normalizes* the trimmed mean signal of the experiment to the trimmed mean signal of the baseline **(Figure E.19)**.

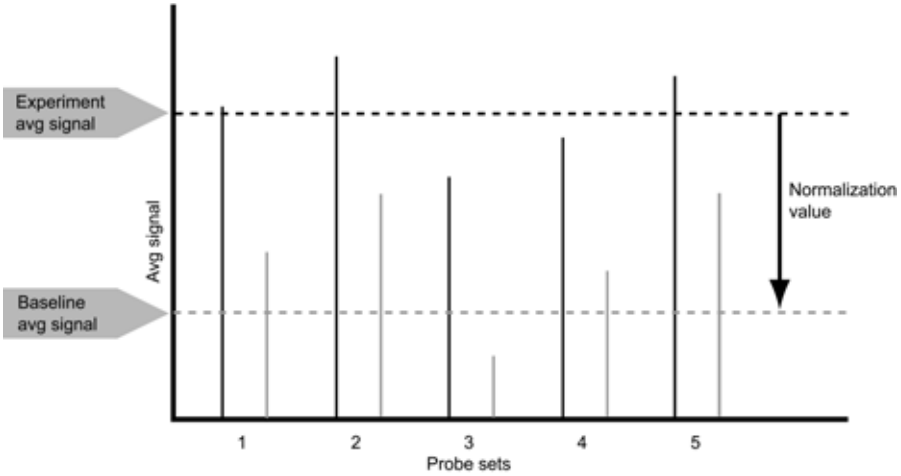


Figure E.19
Normalization Value normalizes the average signal of the experiment to the average signal of the baseline

1. In the Normalization tab, choose the **All Probe Sets** normalization option **(Figure E.20)**.

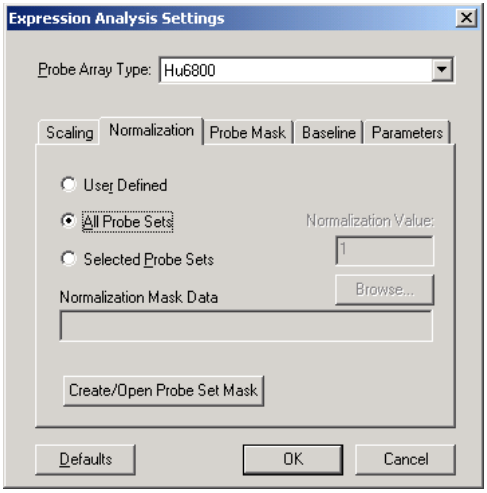


Figure E.20
Expression Analysis Settings, Normalization tab, All Probe Sets normalization option

2. Click **OK** to close the Expression Analysis Settings dialog box.

   The software examines all probe sets on the experiment or baseline array to compute a trimmed mean signal for the experiment and a trimmed mean signal for the baseline.

   It computes a normalization value so that:

   Trimmed Mean $\text{Signal}_{\text{baseline}}$ = (Normalization Value) x (Trimmed Mean $\text{Signal}_{\text{experiment}}$)

Selected Probe Sets Normalization

Selected Probe Sets normalization adjusts or normalizes the trimmed mean signal of the experiment to the trimmed mean signal of the baseline **(Figure E.19)**.

For this normalization option, GCOS utilizes user-selected probe sets (specified by a normalization mask file) to compute the trimmed mean signal of the experiment and baseline, and derive a normalization value so that:

Trimmed Mean $\text{Signal}_{\text{baseline selected probe sets}}$ = Normalization Value x Trimmed Mean $\text{Signal}_{\text{experiment selected probe sets}}$

Creating a Normalization Mask File

1. In the Normalization tab, choose the **Selected Probe Sets** option **(Figure E.21)**.

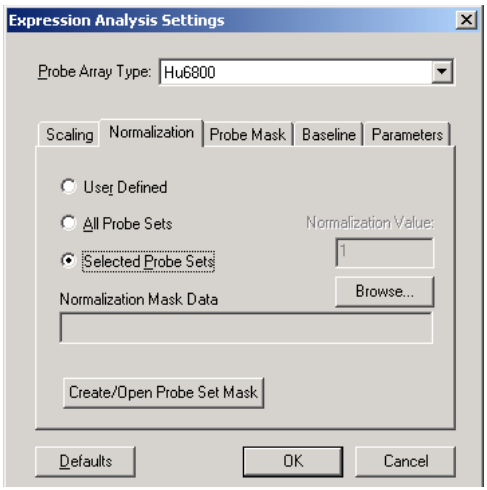


Figure E.21
Expression Analysis Settings, Normalization tab, Selected Probe Sets option

2. Click **Create/Open Probe Set Mask**.

   ⇒ The Probe Set Mask File dialog box appears **(Figure E.22)**.

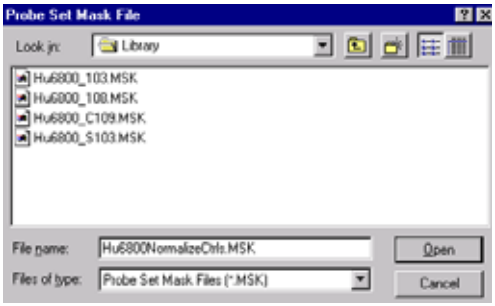


Figure E.22
Probe Set Mask File dialog box

✔ NOTE

The mask files (.msk) include Scale Factor, Normalization, or Probe mask files. Each type of mask file has a different function and is created in a separate tab of the Expression Analysis Settings dialog box.*

3. Enter a name for the new normalization mask file in the **File name** box and click **Open.**

   ⇒ The Probe Set Mask Definition dialog box appears **(Figure E.23)**.

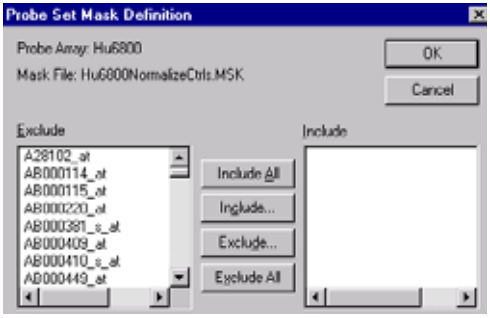


Figure E.23
Probe Set Mask Definition dialog box

4. Highlight the desired probe set names in the **Exclude** list. Click **Include** to add them to the **Include** list for the new *.msk file.

5. To remove a probe set name(s) from the **Include** list, highlight the name(s) in the **Include** list, then click **Exclude**.

   Include All automatically moves all of the probe set names from the **Exclude** list to the **Include** list.

   Exclude All automatically moves all of the probe set names from the **Include** list to the **Exclude** list.

6. Click **OK** when finished to create the normalization mask file and close the Probe Set Mask Definition dialog box.

Selecting a Normalization Mask File

1. In the Normalization tab, click **Browse** **(Figure E.24)**.

   ⇒ The Probe Set Mask File dialog box appears **(Figure E.25)**.

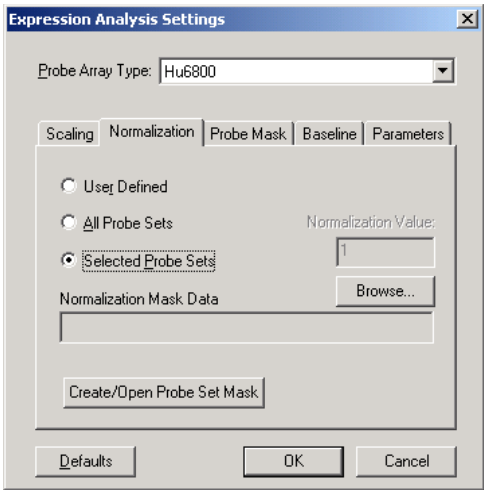


Figure E.24
Expression Analysis Settings, Normalization tab, Selected Probe Sets option

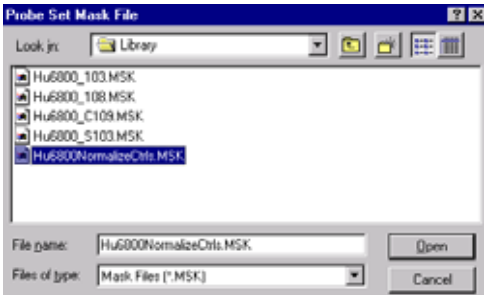


Figure E.25
Probe Set Mask File dialog box

✔ NOTE

Mask files are specific for the probe array type. GCOS will not open a mask file that is incompatible with the currently selected probe array type.

2. Double-click the desired *.msk file.

   ⇒ The Probe Set Mask File dialog box closes and the Expression Analysis Settings dialog box displays the selected *.msk in the **Normalization Mask File** box **(Figure E.26)**.

Figure E.26
Expression Analysis Settings, Normalization tab, normalization mask file selected

Editing a Normalization Mask File

1. In the Normalization tab, choose the **Selected Probe Sets** option **(Figure E.27)**.

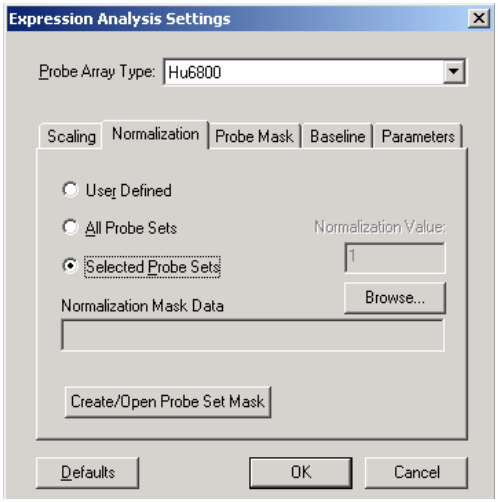


Figure E.27
Expression Analysis Settings, Normalization tab, Selected Probe Sets option

2. Click **Create/Open Probe Set Mask** **(Figure E.27)**.

   ⇒ The Probe Set Mask File dialog box appears **(Figure E.28)**.

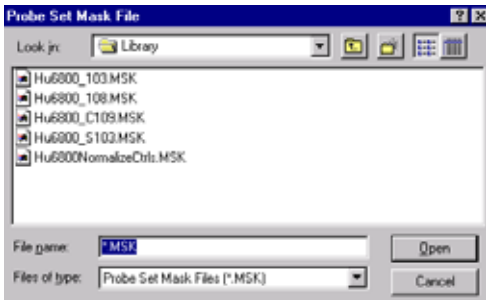


Figure E.28
Probe Set Mask File dialog box

3. Double-click the desired *.msk file.

   ⇒ The Probe Set Mask Definition dialog box appears **(Figure E.29)**.

   The **Include** list displays the probe sets included in the *.msk file. The **Exclude** list displays the probe sets not included in the *.msk file.

Figure E.29
Probe Set Mask Definition dialog box

4. To remove a probe set(s) from the *.msk file:

   a. Highlight the probe set name(s) in the **Include** list.

   b. Click **Exclude**.

5. To add a probe set(s) to the *.msk file:

   a. Highlight the probe set name(s) in the **Exclude** list

   b. Click **Include**.

   Include All automatically moves all of the probe set names from the **Exclude** list to the **Include** list.

   Exclude All automatically moves all of the probe set names from the **Include** list to the **Exclude** list.

6. Click **OK** when finished to close the Probe Set Mask Definition dialog box.

Probe Mask Tab

User-selected probe pairs may be excluded or *masked* from an expression analysis. In the Probe Mask tab **(Figure E.30)**, you can create a probe mask file that specifies the probe pairs to exclude from an analysis.

GCOS also offers advanced features that automatically generate three types of probe masks: cross hybridization, hybridization, or spike mask. See Appendix G, *Cross Hybridization, Hybridization, & Spike Probe Masks*, on page 573 for more information about probe masks.

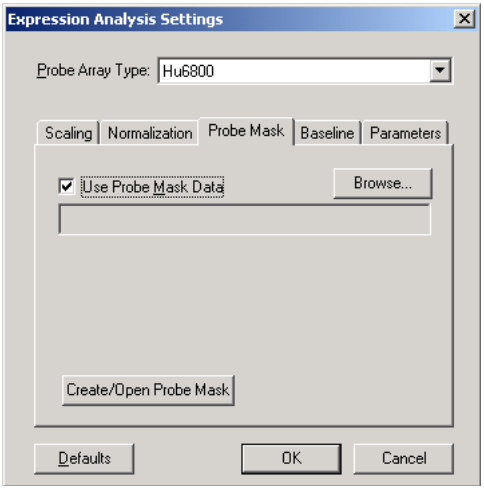


Figure E.30
Expression Analysis Settings, Probe Mask tab

Creating a Probe Mask

1. In the Probe Mask tab, click **Create/Open Probe Mask** **(Figure E.30)**.

   ⇒ The Probe Mask File dialog box appears **(Figure E.31)**.

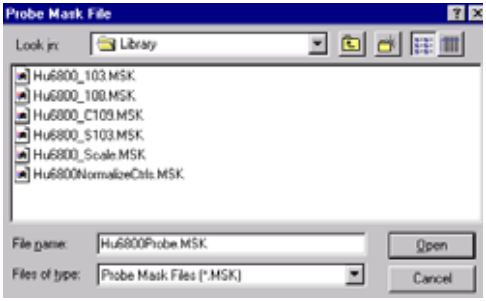


Figure E.31
Probe Mask File dialog box

✔ NOTE

*The *.msk files include Scale Factor, Normalization, or Probe mask files. Each type of mask file has a different function and is created in a separate tab of the Expression Analysis Settings dialog box.*

2. Enter a name for the new probe mask file in the **File name** box and click **Open**.

   ⇒ The Probe Mask Definition dialog box appears **(Figure E.32)** and displays the probe array type and the probe mask name.

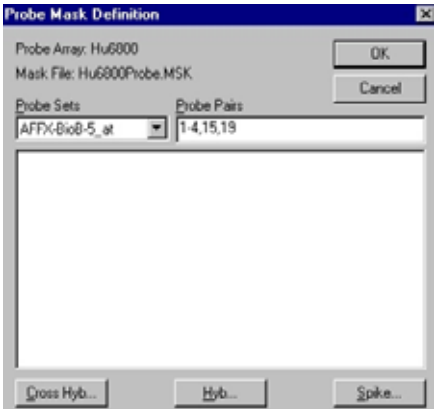


Figure E.32
Probe Mask Definition dialog box

3. Select the desired probe set name from the **Probe Sets** drop-down list and enter the desired probe pair numbers in the **Probe Pairs** field (following the example format: 1-4,7,15,19).

   These probe pairs will be omitted from the analysis.

4. Click the box below the **Probe Sets** drop-down list to add the probe pairs to the probe mask **(Figure E.33)**.

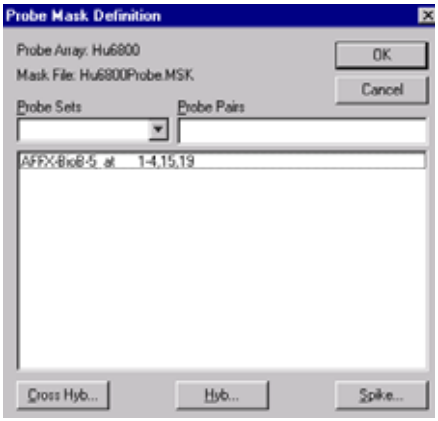


Figure E.33
Probe pairs selected for the probe mask file

5. Repeat step 3 and step 4 to specify additional probe pairs for the probe mask.

6. Click **OK** when finished adding probe pairs to the probe mask.

Selecting a Probe Mask

1. In the Probe Mask tab, choose the **Use Probe Mask File** option **(Figure E.34)**.

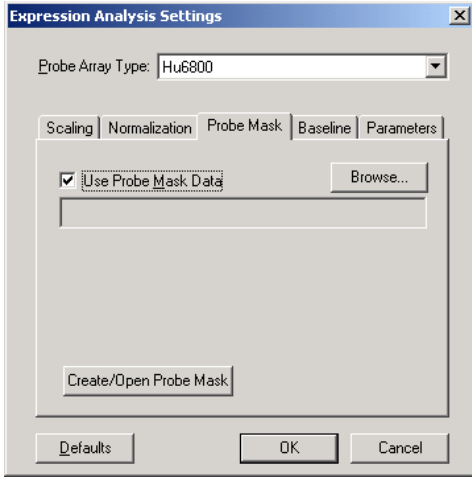


Figure E.34
Expression Analysis Settings, Probe Mask tab

2. Click **Browse**.

   ⇒ The Probe Mask File dialog box appears **(Figure E.35)**.

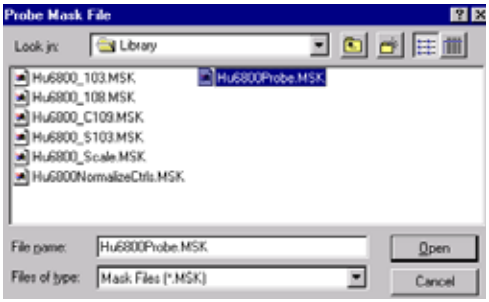


Figure E.35
Probe Mask File dialog box

3. Double-click the desired probe mask.

   ⇒ The Probe Mask tab displays the selected probe mask **(Figure E.36)**.

✔ NOTE

Mask files are specific for the probe array type. GCOS will not open a mask file that is incompatible with the currently selected probe array type.

Figure E.36
Expression Analysis Settings, Probe Mask tab

Editing a Probe Mask

1. In the Probe Mask tab, click **Create/Open Probe Mask** **(Figure E.37)**.

   ⇒ The Probe Mask File dialog box appears **(Figure E.38)**.

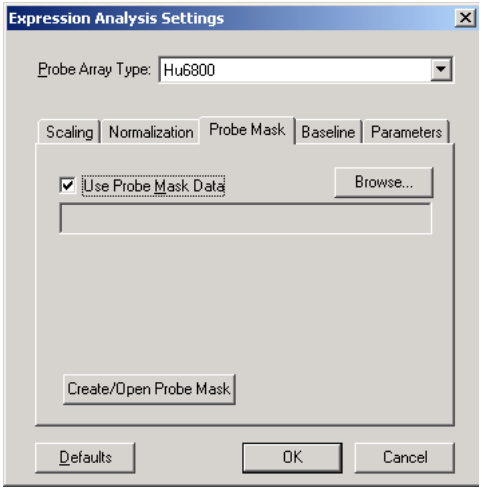


Figure E.37
Expression Analysis Settings

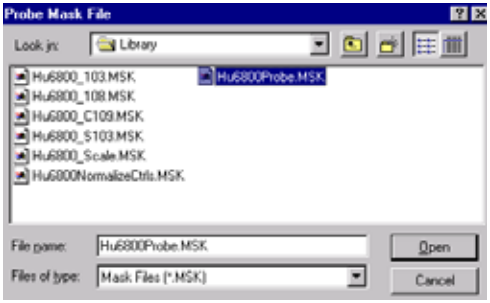


Figure E.38
Probe Mask File dialog box

2. Click the desired probe mask file and click **OK** (or double-click the file name).

   ⇒ The Probe Mask Definition dialog box displays the probe pairs in the selected probe mask **(Figure E.39)**.

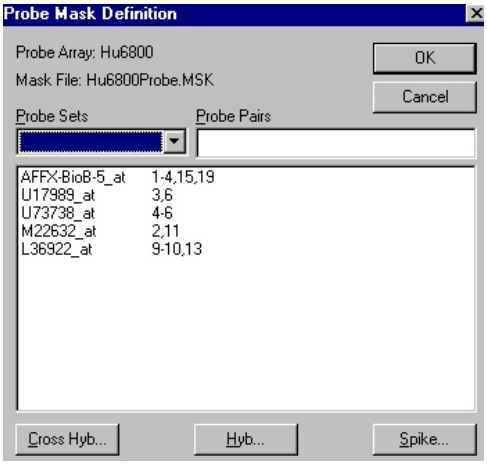


Figure E.39
Probe Mask Definition dialog box

3. Click the probe set/probe pair entry you wish to edit.

   ⇒ The Probe Sets and Probe Pairs box automatically display the selection **(Figure E.40)**.

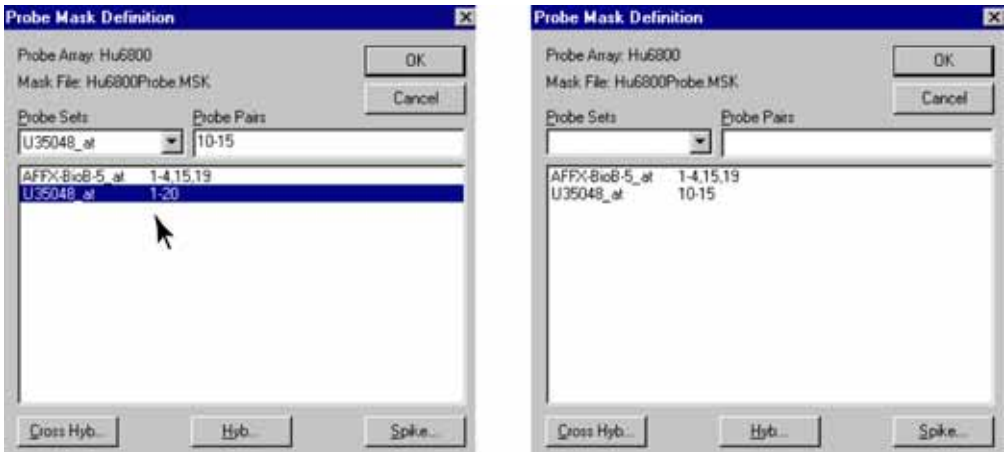


Figure E.40
Edit entries in the Probe Pairs box and click the lower box (left) to update the probe pair list (right)

4. Edit or delete the entry in the Probe Pairs field, then click the lower field to update the list of probe pairs **(Figure E.40)**.
5. Click **OK** when finished to close the Probe Mask Definition dialog box.

Baseline Tab

In the baseline tab, the user selects the analysis output file (*.chp) that will serve as the baseline in a comparison expression analysis **(Figure E.41)**.

✔ NOTE

Do not choose the **Use Baseline Comparison Data** *option for an single-array analysis.*

1. In the Baseline tab, choose the **Use Baseline Comparison Data** option **(Figure E.41)**.

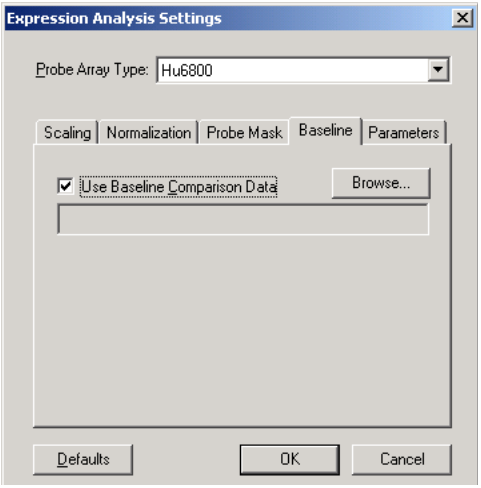


Figure E.41
Expression Analysis Settings, Baseline tab

2. Click **Browse**.

   ⇒ The Baseline Comparison File dialog box appears **(Figure E.42)**.

✔ NOTE

The baseline file must be derived from the same type of probe array selected in the **Probe Array Type** *drop-down list in the Expression Analysis Settings dialog box* **(Figure E.41)**.

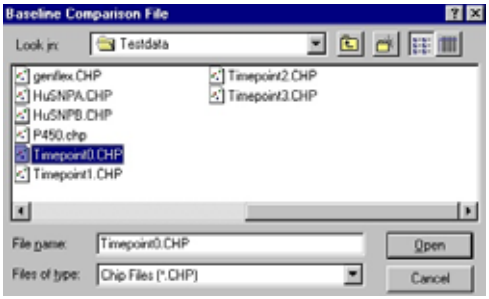


Figure E.42
Baseline Comparison File dialog box

3. Double-click the baseline *.chp and click **Open**.

   The Baseline tab displays the selected baseline file for comparison analyses **(Figure E.43)**.

Figure E.43
Expression Analysis Settings, Baseline tab

Parameters Tab

The Parameters tab displays user-modifiable Statistical Expression algorithm parameters **(Figure E.44)**. **Table E.3** describes the parameters.

✔ NOTE

Versions of Microarray Suite lower than 5.0 run the Empirical Expression algorithm. (See Expression Analysis Metrics, on page 524 for more information about the Empirical Expression algorithm.)

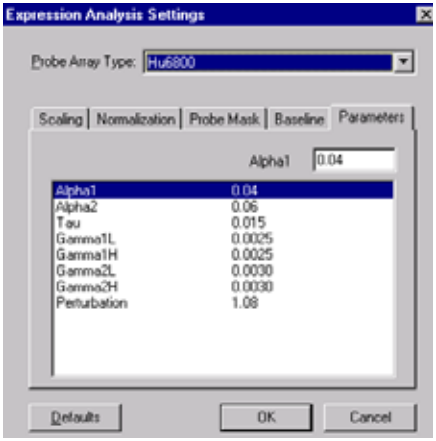


Figure E.44
Expression Analysis Settings, Parameters tab

The parameter default values were determined through extensive empirical testing at Affymetrix. Changing a parameter value affects the:

- algorithm output (see Appendix , *Expression Analysis Metrics*, on page 524 for more information about the Statistical and Empirical Expression algorithm outputs)
- assay sensitivity (ability to make true Detection or Change calls)
- assay specificity (ability to minimize false Detection or Change calls)

Table E.3
User-modifiable Statistical Expression algorithm parameters

Parameter	Description	Effect of Changing Parameter
Alpha1 (α_1)	The *p*-value threshold that defines Present and Marginal Detection calls. If: $p < \alpha_1$ the call is Present $\alpha_1 \leq p < \alpha_2$ the call is Marginal	Decreasing α_1 may reduce the number of false Present calls (increases specificity) and the number of true Present calls (decreases sensitivity).
Alpha2 (α_2)	The *p*-value threshold that defines Marginal and Absent Detection calls. If: $\alpha_1 \leq p < \alpha_2$ the call is Marginal $p \geq \alpha_2$ the call is Absent	Increasing α_2 may reduce the number of false Absent calls (increases sensitivity) and the number of true Absent calls (decreases specificity).
Tau (τ)	Threshold for the probe set discrimination score. If the discrimination score $< \tau$, the difference between the PM and MM cells in the probe set is considered insignificant. The algorithm does not make a call for the probe set.	Increasing τ may reduce the number of false Present calls (increases specificity) and the number of true Present calls (decreases sensitivity).
Gamma1L (γ_{1L})	The lower limit of γ_1 which is the *p*-value threshold that defines Increase and Marginal Increase Change calls.	Decreasing γ_{1L} and γ_{1H} decreases γ_1. Decreasing γ_1 may reduce the number of false Increase and Decrease calls (increases specificity) and may also reduce the number of true Increase and Decrease calls (decreases sensitivity).
Gamma1H (γ_{1H})	The upper limit of γ_1 which is the *p*-value threshold that defines Increase and Marginal Increase Change calls.	
Gamma2L (γ_{2L})	The lower limit of γ_2 which is the *p*-value threshold that defines Marginal Increase and No Change calls.	Increasing γ_{2L} and γ_{2H} increases γ_2. Increasing γ_2 may reduce the number of false No Change calls (increases specificity) and may also reduce the number of true No Change calls (decreases sensitivity).
Gamma2H (γ_{2H})	The upper limit of γ_2 which is the *p*-value threshold that defines marginal, No Change and Change calls.	
Perturbation (d)	Determines the spread between the three normalization factors the algorithm computes for an experiment and baseline in a comparison analysis.	Increasing d may increase the number of true No Change calls (increases sensitivity) and may also increase the number of false No Change calls (decreases specificity).

Changing a Parameter Value

1. In the Parameters tab and make a selection from the **Probe Array Type** drop-down list.
2. Click the parameter you wish to change.

   ⇒ The parameter box displays the current value **(Figure E.45)**.

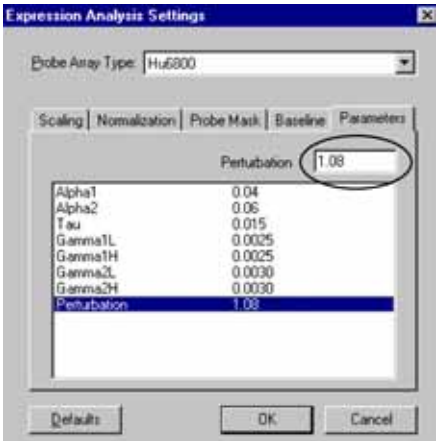
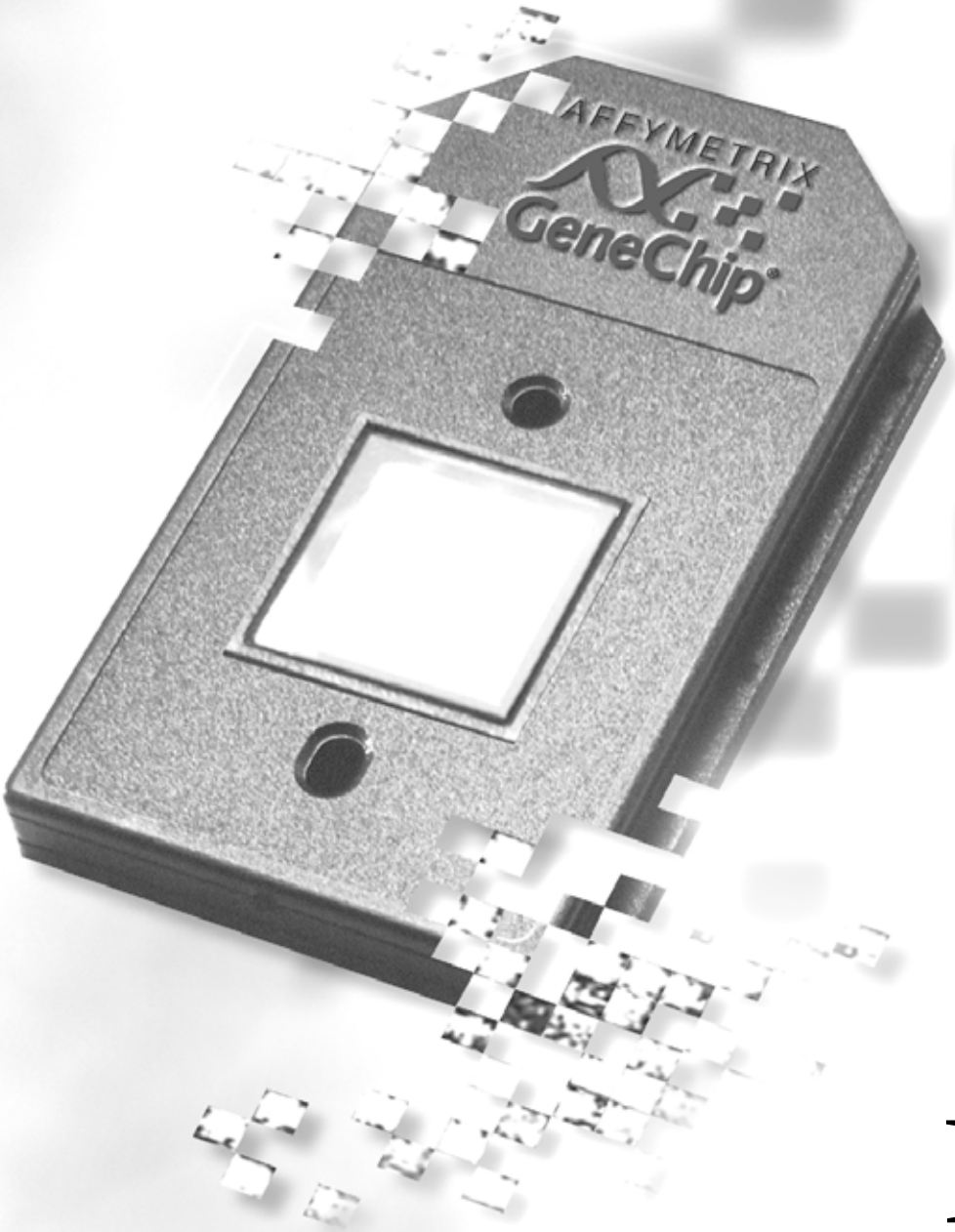


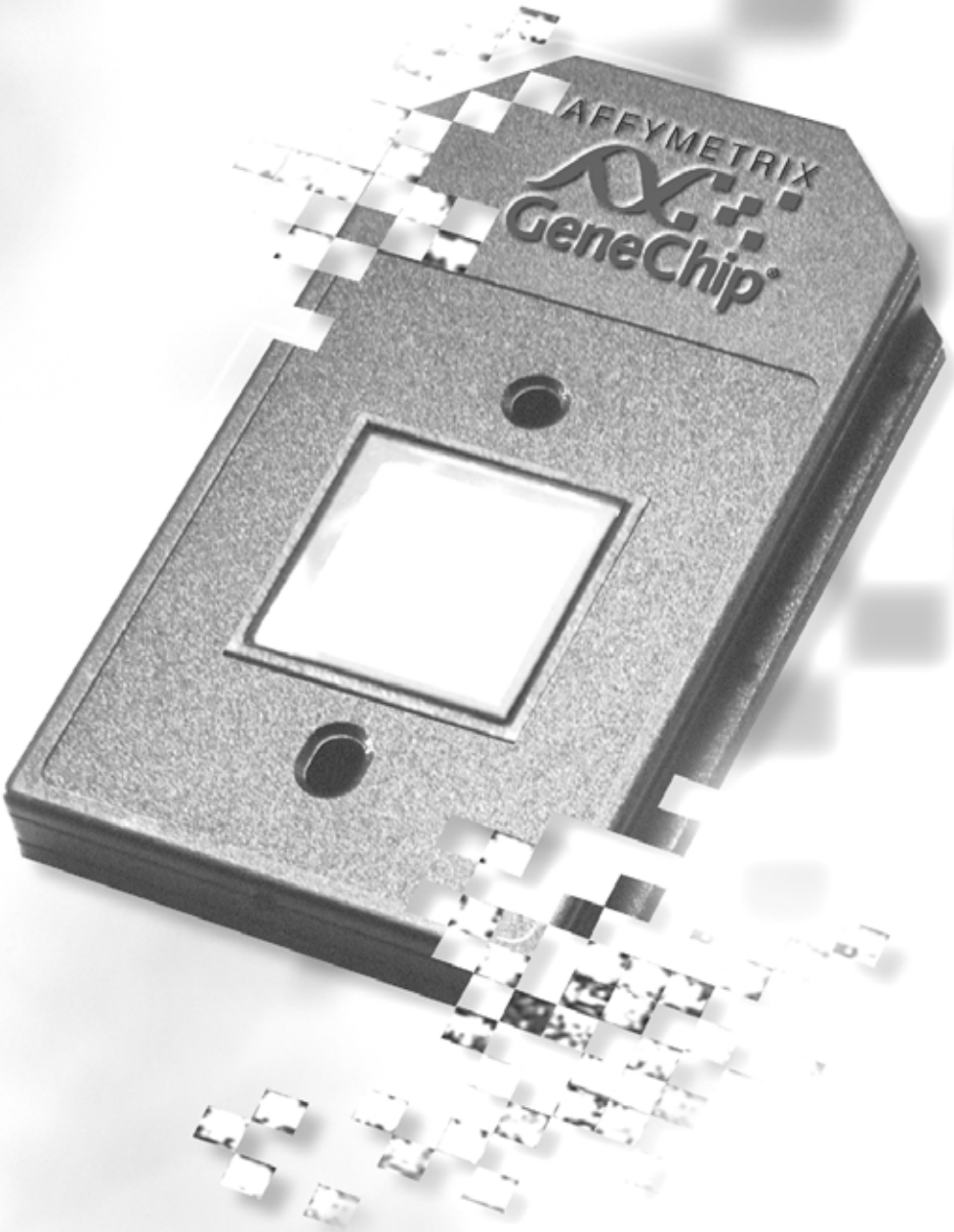
Figure E.45
Expression Analysis Settings, Parameters tab

3. Enter a new value for the parameter in the parameter box.
4. Click **OK** to close the Expression Analysis Settings dialog box.



F

Appendix F



Probe Information (Library) Data

The probe information or *library* data includes the probe array design characteristics, probe utilization and content, and scanning and analysis parameters. Library data are unique to each probe array type. Library data includes user-defined mask files (see **Table F.1**). The default path for the library directory is <servername>:\GeneChip\Affy_Data\Library.

Table F.1
User-defined mask files

Mask File Name	File Extension	Description
Probe Mask	.msk	A user-specified list of probe pairs that are excluded from an analysis.
Cross Hybridization Probe Mask	.msk	A type of probe mask that specifies probe pairs that include a PM or MM probe cell whose intensity exceeds a user-specified limit. These probe pairs will be excluded from the analysis.
Hybridization Probe Mask	.msk	A type of probe mask that specifies probe pairs where PM – MM < Difference Threshold or PM/MM < Ratio Threshold. These probe pairs will be excluded from the analysis.
Spike Probe Mask	.msk	A type of probe mask that specifies probe pairs where: $(PM - MM)_{spike} - (PM - MM)_{unspike}$ < Difference Threshold or $(PM\text{-}MM)_{spike}/(PM - MM)_{unspike}$ < 1 + Ratio Threshold. These probe pairs will be excluded from the analysis.

Fluidics Protocol Data

The fluidics protocol data include the instrument control instructions used by the GeneChip® fluidics station. Fluidics protocols are written to a directory that is specified during GCOS installation. The default path for the fluidics protocols is <servername>:\GeneChip\Affy_Data\Protocol.

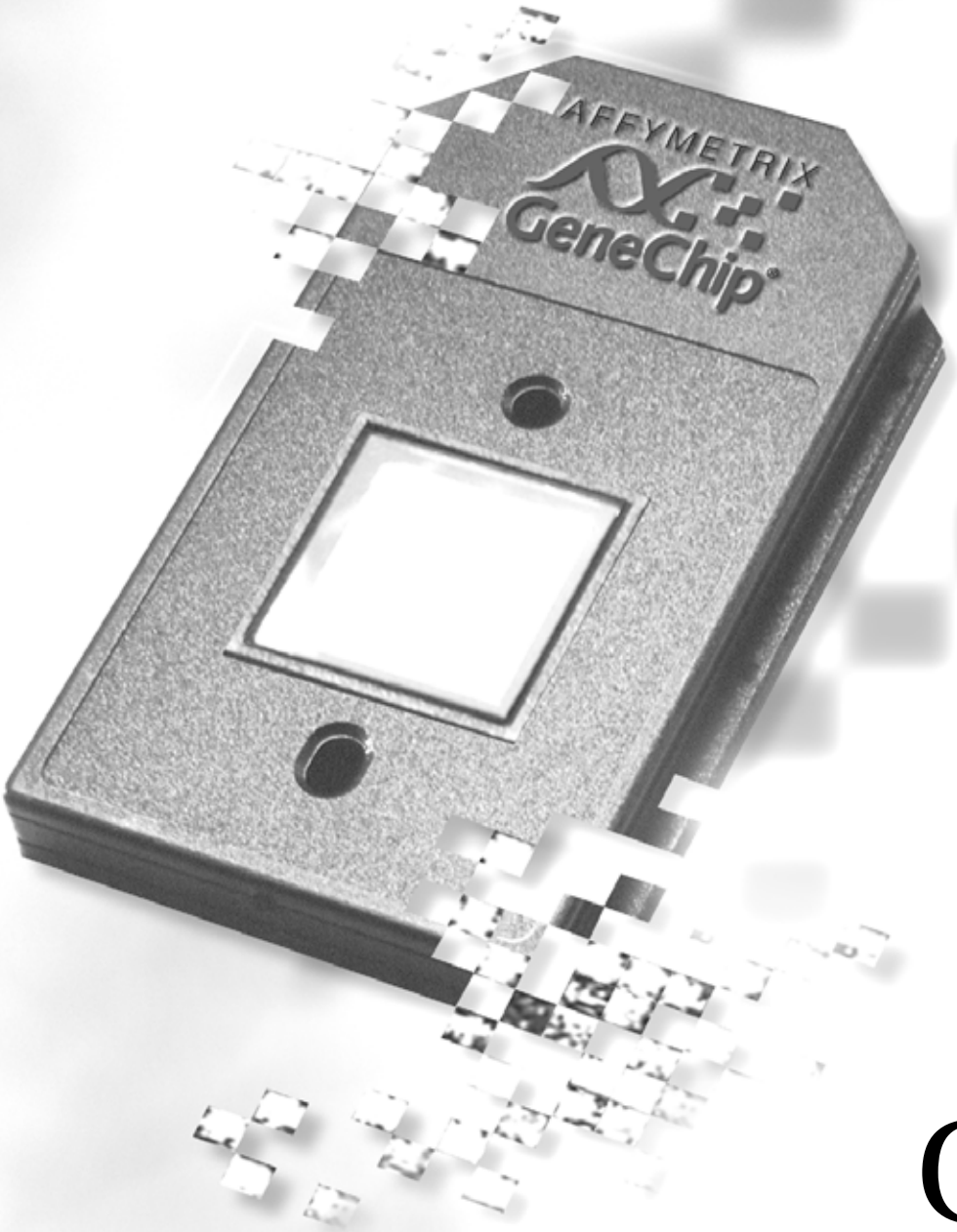
Experiment Data

During experiment setup, you enter information to register the sample and define the experiment. The software generates the other experiment data (image, cell intensities, probe analysis data) as the analysis proceeds. Experiment data are written to a directory that is specified during GCOS installation. The default path for experiment data is <servername>:\GeneChip\Affy_Data\Data\.

GCOS stores the experiment data in the process database, a relational database that maintains associations between experiments, samples, and the experiment data generated during analysis. **Table F.2** explains the different types of experiment data in the process database.

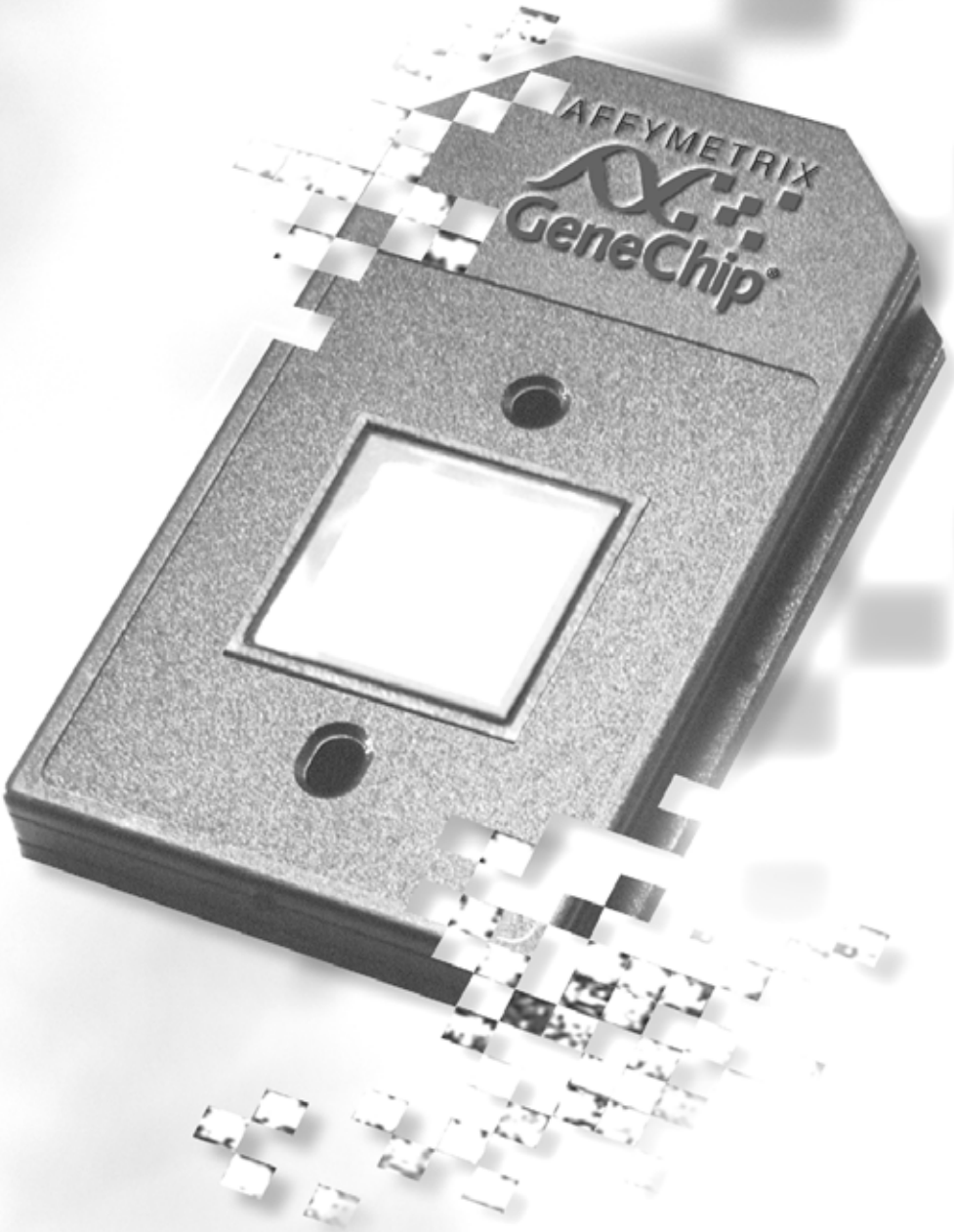
Table F.2
Experiment data files

Experiment Data Name	Data Description
Experiment	Information about the experiment name, sample, and probe array type. The experiment name also provides the default name for subsequent data that are generated during experiment analysis.
Image Data	Image of the scanned probe array (.dat file) and information about the experiment & sample that the image pertains to.
Cell Intensities	Single intensity value for each probe cell delineated by the grid (.cel file calculated by the Cell Analysis algorithm) and information about the: 1) image that the cell intensities were derived from, and 2) experiment and sample that the image pertains to.
Probe Analysis (chip data)	Output generated from the analysis of a probe array (.chp file) and information about the: 1) cell intensities for the analysis, 2) image that the cell intensities were derived from, and 3) experiment and sample that the image pertains to.
Report	Report generated from the analysis output file (.chp) and information about the: 1) analysis output file (.chp) the report was derived from, 2) cell intensities for the analysis, 3) image that the cell intensities were derived from, and 4) experiment and sample that the image pertains to
Data	A standard format for text files. GCOS exports text in this file format. A standard format for Excel files. GCOS export text in this file format.



G

Appendix G



Cross Hybridization, Hybridization, & Spike Probe Masks

A probe mask specifies probe pairs that are to be excluded from an expression analysis. There are three types of probe masks:

- Cross hybridization probe mask specifies probe pairs that include a perfect match (PM) or mismatch (MM) probe cell intensity greater than a user-specified limit.
- Hybridization probe mask includes probe pairs that meet either of the following criteria:

  $$PM - MM \leq \text{Difference Threshold}$$

  or

  $$PM/MM \leq \text{Ratio Threshold}$$

- Spike probe mask includes probe pairs that meet either of the following criteria:

  $$(PM - MM)_{spike} - (PM - MM)_{unspike} < \text{Difference Threshold},$$

  or

  $$(PM - MM)_{spike} / (PM - MM)_{unspike} < 1 + \text{Ratio Threshold}$$

Viewing the Probe Mask Tab

You can create, view, or edit probe masks in the Probe Mask tab of the Expression Analysis Settings dialog box.

To view the probe mask tab:

1. Click **Analysis Settings** in the shortcut bar, then click **Expression**. Alternatively, select **Tools →Analysis Settings →Expression** from the menu bar.

   ⇒ The Expression Analysis Settings dialog box appears **(Figure G.1)**.

2. Click the Probe Mask tab.

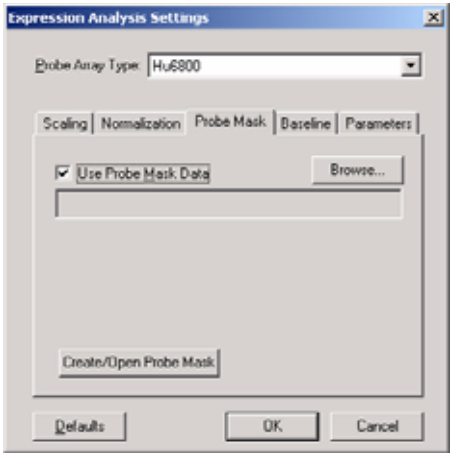


Figure G.1
Expression Analysis Settings, Scaling tab, User Defined scaling option selected

3. Make a selection from the **Probe Array Type** drop-down list.

   The settings are specific for the selected probe array type and do not affect the settings for other types of probe arrays.

Cross Hybridization Probe Mask

GCOS analyzes one or more user-specified .cel files to generate a cross hybridization probe mask. The cross hybridization probe mask specifies probe pairs that have a perfect match (PM) or mismatch (MM) probe cell with an intensity that exceeds a user-specified limit.

A series of .cel files may be analyzed and the results from each analysis may be combined in an *And* (intersection) or *Or* (union) fashion to create a composite cross hybridization probe mask. The probe pairs specified by the cross hybridization probe mask are excluded or *masked* from an expression analysis.

Creating a Cross Hybridization Probe Mask

1. In the shortcut bar, click **Analysis Settings**, then click **Expression**.
   Alternatively, select **Tools →Analysis Settings →Expression** from the menu bar.
   ⇒ The Expression Analysis Settings dialog box appears **(Figure G.2)**.

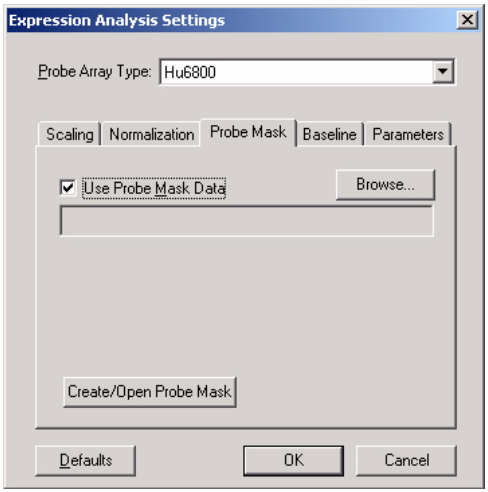


Figure G.2
Expression Analysis Settings, Probe Mask tab

2. Click the Probe Mask tab
3. Click **Create/Open Probe Mask**.

   ⇒ The Probe Mask File dialog box appears **(Figure G.3)**.

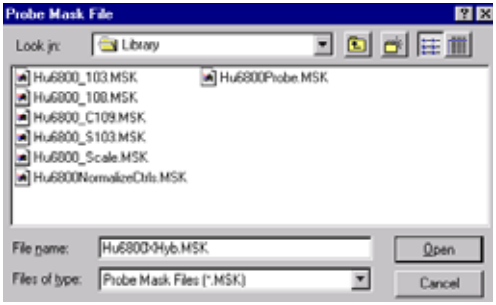


Figure G.3
Probe Mask File dialog box

4. Enter a name for the new mask in the **File Name** box and click **Open**.

   ⇒ The Probe Mask Definition dialog box appears **(Figure G.4)**.

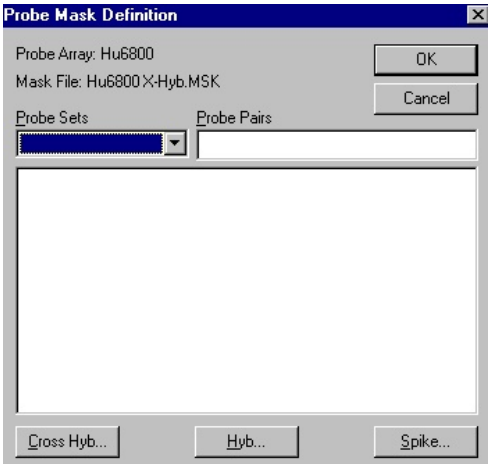


Figure G.4
Probe Mask Definition dialog box

5. Click **Cross Hyb**.

   ⇒ The Cross Hybridization dialog box appears **(Figure G.5)**.

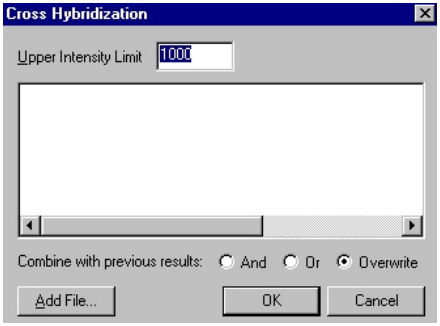


Figure G.5
Cross Hybridization dialog box

6. Enter a new upper intensity limit (if necessary).
7. Click **Add File.**

   ⇒ This displays the Open dialog box **(Figure G.6)**.

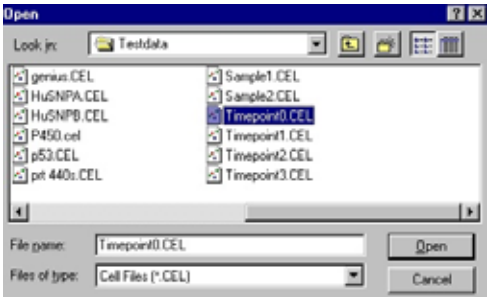


Figure G.6
Open dialog box displays .cel files available for the cross hybridization probe mask

8. Double-click a .cel file in the Open dialog box.

   ⇒ This adds the selected .cel to the Cross Hybridization dialog box **(Figure G.7)**.

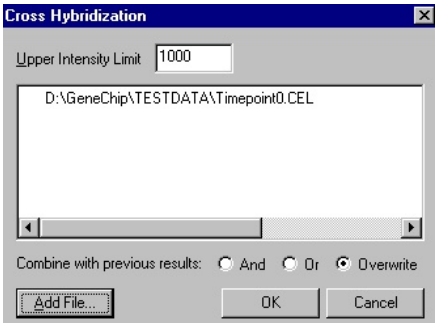


Figure G.7
Cross Hybridization dialog box displays .cel files selected for the cross hybridization probe mask

9. Repeat step 7 and step 8 until all desired .cel files have been added to the Cross Hybridization dialog box.

   GCOS identifies the probe pairs in each .cel file that include a PM or MM probe cell with an intensity greater than the user-specified Upper Intensity Limit (the default is 1000).

   The results from each .cel file may be combined in an *And* (intersection) or *Or* (union) fashion to create the cross hybridization probe mask.

10. Double-click the second (and any subsequent) .cel file name to toggle between the **And** and **Or** option **(Figure G.8)**.

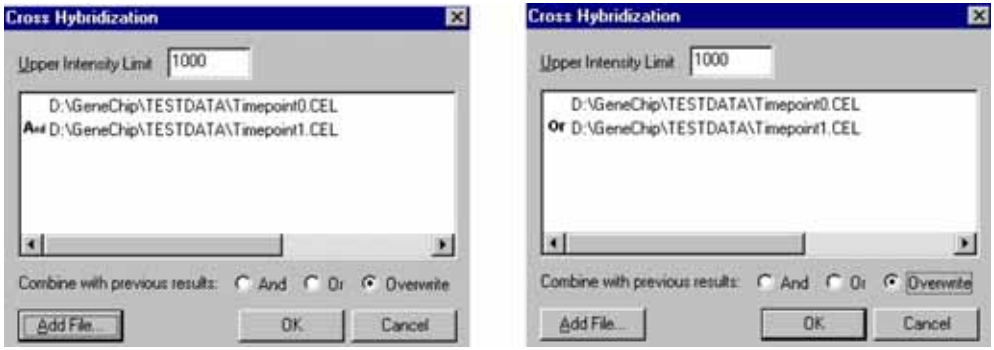


Figure G.8
And combined .cel files (left), *Or* combined .cel files (right)

11. Click **OK** to generate the cross hybridization probe mask.

    ⇒ The Probe Mask Definition dialog box displays a list of the probe pairs in the cross hybridization probe mask **(Figure G.9)**.

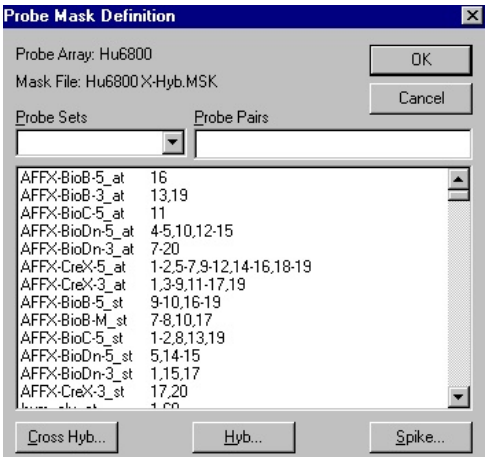


Figure G.9
Probe pairs in the Cross Hybridization Probe mask

12. Click OK to close the Probe Mask Definition dialog box.

Editing a Cross Hybridization Probe Mask

1. In the Probe Mask tab, click **Create/Open Probe Mask** **(Figure G.10)**.

   ⇒ The Probe Mask File dialog box appears **(Figure G.11)**.

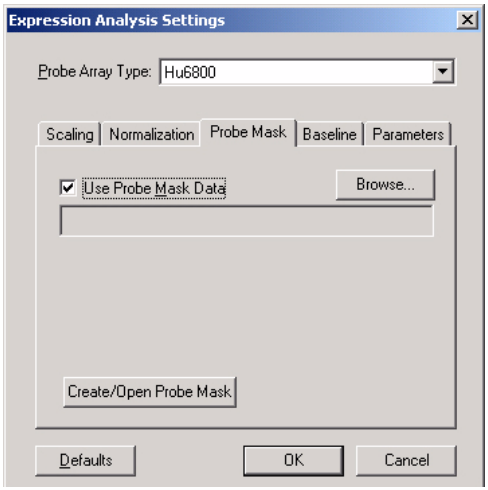


Figure G.10
Expression Analysis Settings dialog box, Probe Mask tab

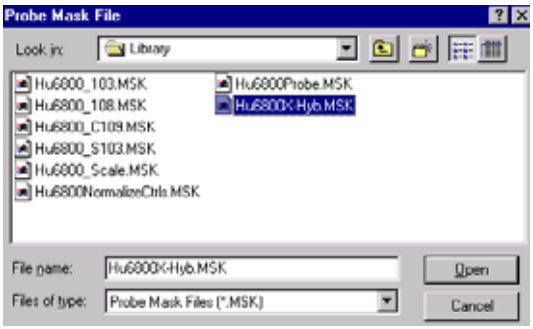


Figure G.11
Probe Mask File dialog box

2. Double-click the cross hybridization probe mask file (.msk) you wish to edit **(Figure G.11)**.

   ⇒ The Probe Mask Definition dialog box displays the probe pairs in the currently selected probe mask **(Figure G.12)**.

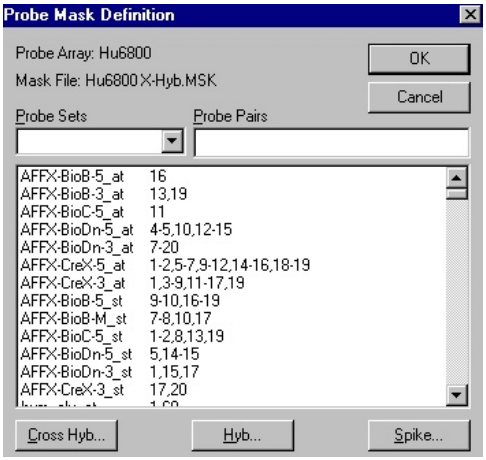


Figure G.12
Probe Mask Definition dialog box

3. In the lower box, click the Probe Set/Probe Pair entry you wish to edit.

   ⇒ The **Probe Sets** box and **Probe Pairs** box automatically display the selection **(Figure G.13)**.

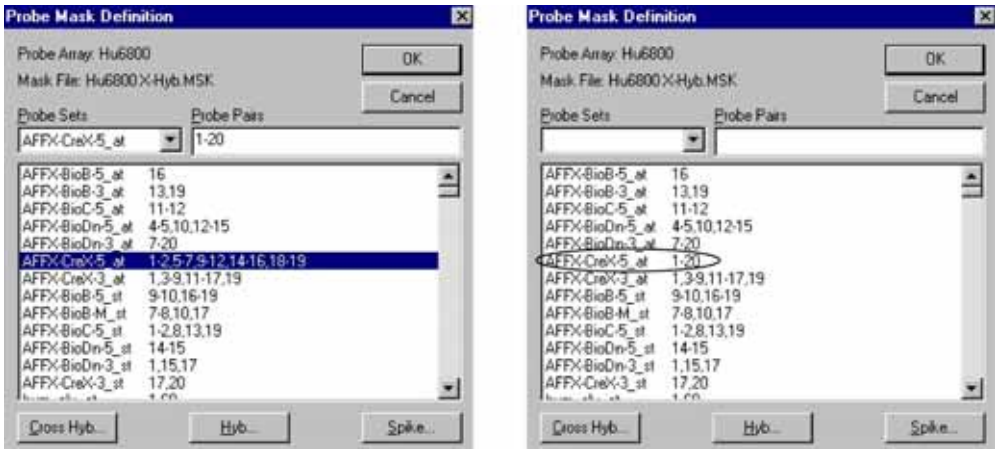


Figure G.13
Edit the Probe Pairs box and then click the lower box (left) to update the probe pair list (right)

4. Edit or delete the entry in the **Probe Pairs** field.
5. Click the lower field to update the list of probe pairs **(Figure G.13)**.
6. Click **OK** when finished to close the Probe Mask Definition dialog box.

Combining Cross Hybridization Probe Masks

A cross hybridization mask may be combined with (*And* or *Or* fashion) or overwrite an existing cross hybridization mask.

1. In the Probe Mask tab, click **Create/Open Probe Mask** **(Figure G.14)**.
   ⇒ The Probe Mask File dialog box appears **(Figure G.15)**.

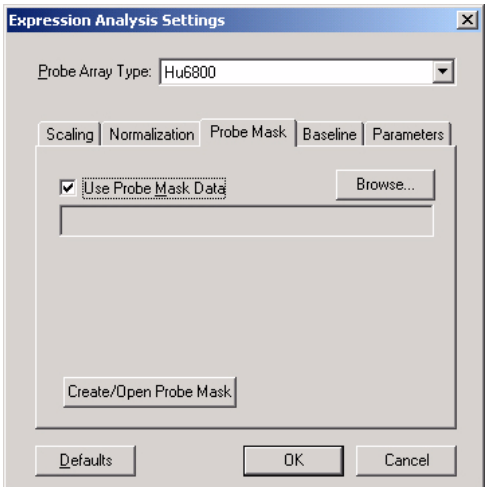


Figure G.14
Expression Analysis Settings dialog box, Probe Mask tab

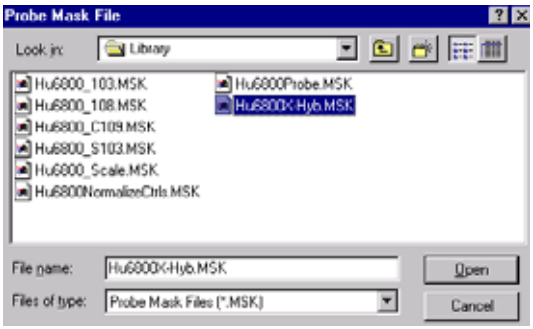


Figure G.15
Probe Mask File dialog box

2. In the Probe Mask File dialog box, double-click the cross hybridization probe mask you wish to combine with another cross hybridization probe mask(s) **(Figure G.15)**.

   ⇒ The Probe Mask Definition dialog box appears **(Figure G.16)**.

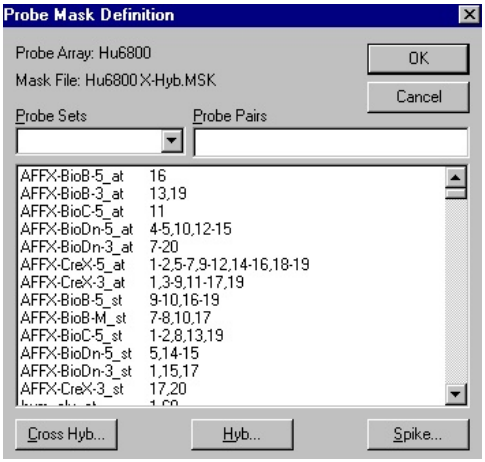


Figure G.16
Probe Mask Definition dialog box

3. Click **Cross Hyb**.

   ⇒ The Cross Hybridization dialog box appears **(Figure G.17)**.

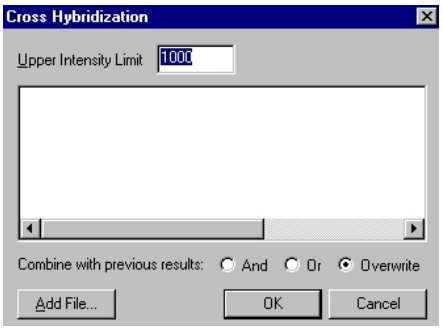


Figure G.17
Cross Hybridization dialog box

4. Enter a new **Upper Intensity Limit** if desired.
5. Click **Add File.**

   ⇒ This displays the Open dialog box **(Figure G.18)**.

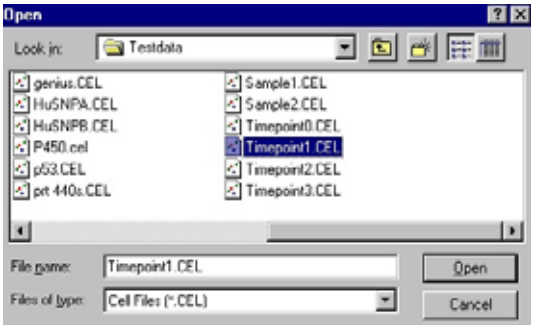


Figure G.18
Open dialog box

6. Double-click the .cel file you wish to combine with the cross hybridization mask selected in step 2.

   ⇒ The Cross Hybridization dialog box displays the selected .cel file **(Figure G.19)**.

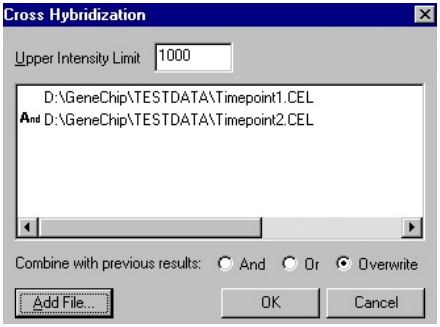


Figure G.19
Cross Hybridization dialog box

7. Click **OK** and repeat step 5 and step 6 to add additional .cel files to the Cross Hybridization dialog box.

8. Double-click the second (and any subsequent) .cel file name to toggle between *And* and *Or* **(Figure G.19)**.

9. Choose a **Combine with previous results** option (And, Or, or Overwrite), then click **OK**.

   GCOS analyzes the selected .cel files and identifies all probe pairs with a PM or MM probe cell that has an intensity greater than the user-specified **Upper Intensity Limit**. It combines the results from the .cel files (And or Or or Overwrite) to create a composite cross hybridization probe mask.

   This composite probe mask is combined with the probe mask selected in step 2 (for example, Hu6800X-hyb.MSK in **Figure G.15**) as specified in step 9 (*And* or *Or* fashion, or overwrites the selected cross hybridization probe mask).

Hybridization Probe Mask

GCOS analyzes one or more user-specified cell intensity files (.cel) to generate a hybridization probe mask comprised of probe pairs that meet either of the following criteria:

PM – MM $\leq$ Difference Threshold

or

PM/MM $\leq$ Ratio Threshold

The Difference and Ratio Thresholds are user-modifiable with defaults of 30 and 1.5 respectively. See Appendix E, *Expression Algorithm, Expression Analysis Metrics & Settings*, on page 515 for more information about the Difference Threshold and Ratio Threshold.

You can analyze a series of .cel files and combine the results from each analysis in an *And* (intersection) or *Or* (union) fashion to create a composite hybridization probe mask.

Creating a Hybridization Probe Mask

1. In the Probe Mask tab, click **Create/Open Probe Mask** **(Figure G.20)**.

   ⇒ The Probe Mask File dialog box appears **(Figure G.21)**.

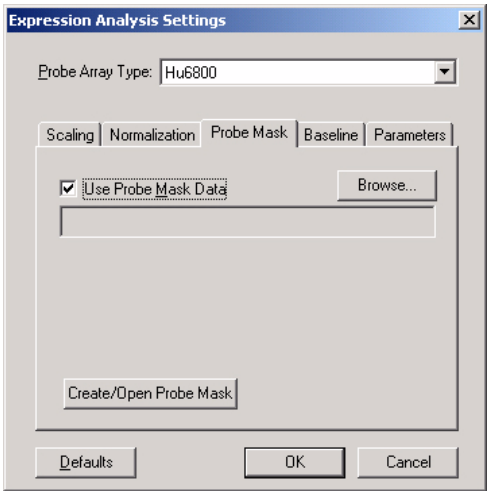


Figure G.20
Expression Analysis Settings dialog box, Probe Mask tab

Figure G.21
Probe Mask File dialog box

2. Enter a name for the new hybridization probe mask in the **File Name** box, then click **Open**.

   ⇒ The Probe Mask Definition dialog box appears **(Figure G.22)**.

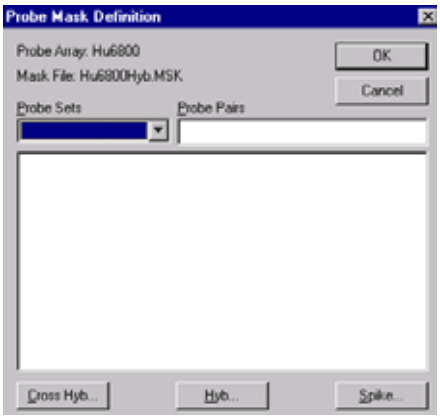


Figure G.22
Probe Mask Definition dialog box

3. Click **Hyb.**

   ⇒ The Hybridization Behavior dialog box appears **(Figure G.23)**.

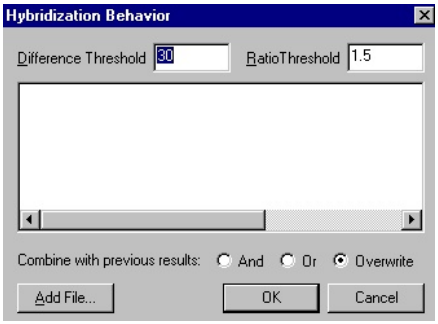


Figure G.23
Hybridization Behavior dialog box

4. Click **Add File**.

   ⇒ This displays the Open dialog box **(Figure G.24)**.

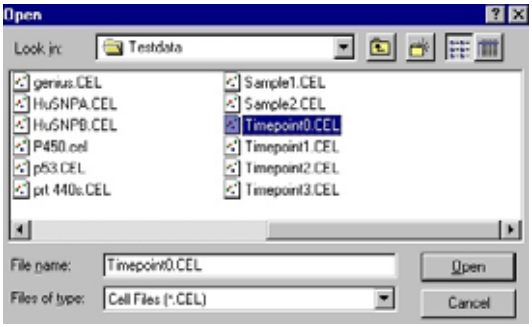


Figure G.24
Open dialog box

5. Double-click the .cel file of interest.

   ⇒ This adds the selected .cel file to the Hybridization Behavior dialog box **(Figure G.25)**.

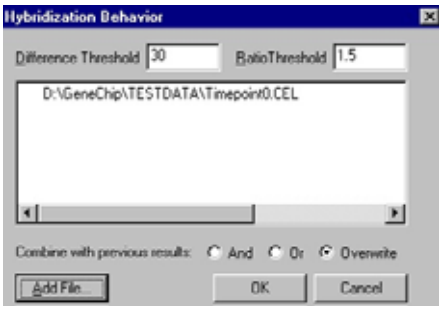


Figure G.25
Hybridization Behavior dialog box

6. If desired, click **Add File** again to select and add another .cel file to the Hybridization Behavior dialog box. Repeat step 4 and step 5 until all desired .cel files have been added.

You can combine the results from the analysis of each .cel file in an *And* (intersection) or *Or* (union) fashion to create the hybridization probe mask.

7. Double-click the second (and each subsequent) .cel file name to toggle between the *And* and *Or* option **(Figure G.26)**.

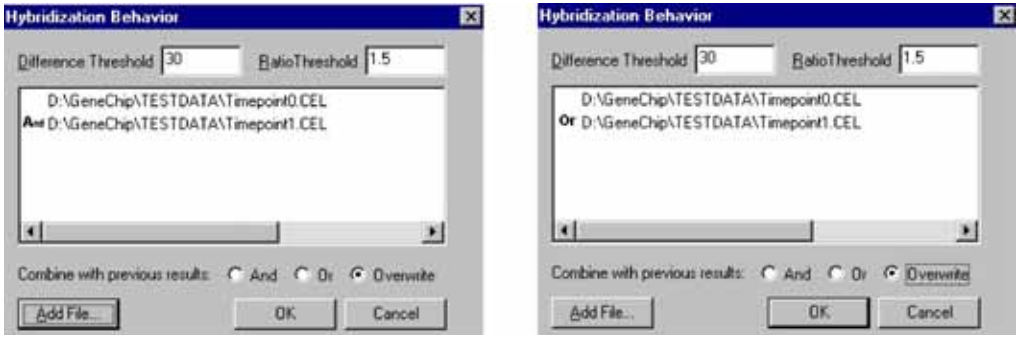


Figure G.26
And combined .cel files analysis results (left), *Or* combined .cel file analysis results (right)

8. Click **OK** to generate the hybridization probe mask.

   ⇒ The Probe Mask Definition dialog box displays the probe pairs of the Hybridization probe mask **(Figure G.27)**.

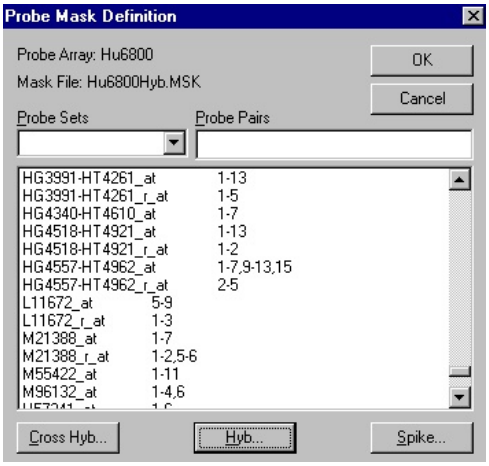


Figure G.27
Probe pairs in the hybridization probe mask

Editing a Hybridization Probe Mask

1. In the Probe Mask tab, click **Create/Open Probe Mask** **(Figure G.28)**.

   ⇒ The Probe Mask File dialog box appears **(Figure G.29)**.

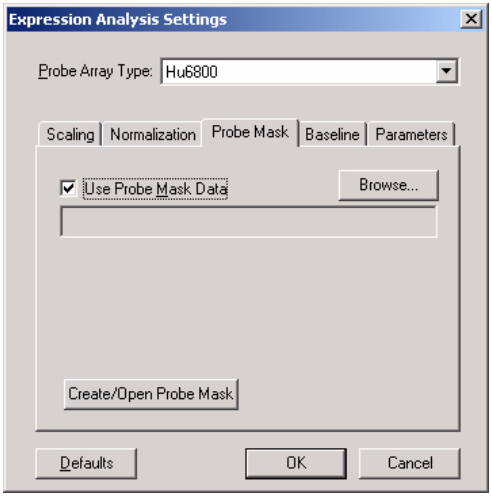


Figure G.28
Expression Analysis Settings dialog box, Probe Mask tab

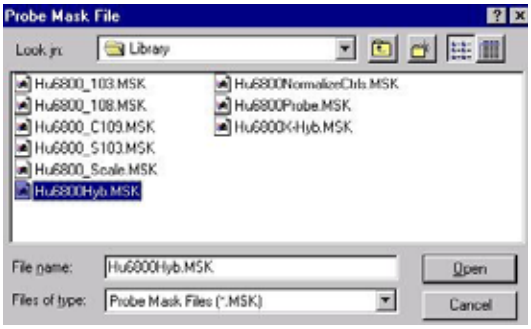


Figure G.29
Probe Mask File dialog box

2. Double-click the hybridization probe mask (.msk) of interest **(Figure G.29)**.

   ⇒ The Probe Mask Definition dialog box displays the probe pairs in the selected hybridization probe mask **(Figure G.30)**.

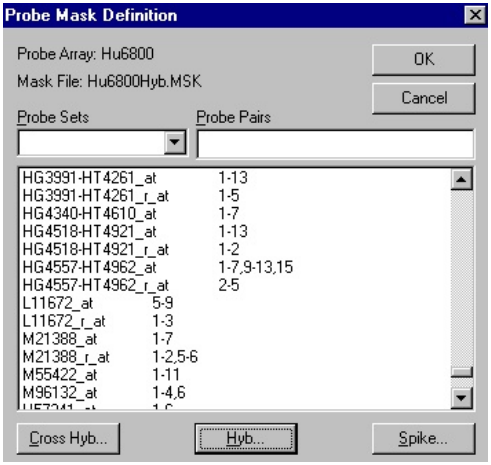


Figure G.30
Probe Mask Definition dialog box

3. In the lower box, click the desired Probe Set/Probe Pair entry you wish to edit.

   ⇒ The **Probe Sets** box and **Probe Pairs** box automatically display the selection **(Figure G.31)**.

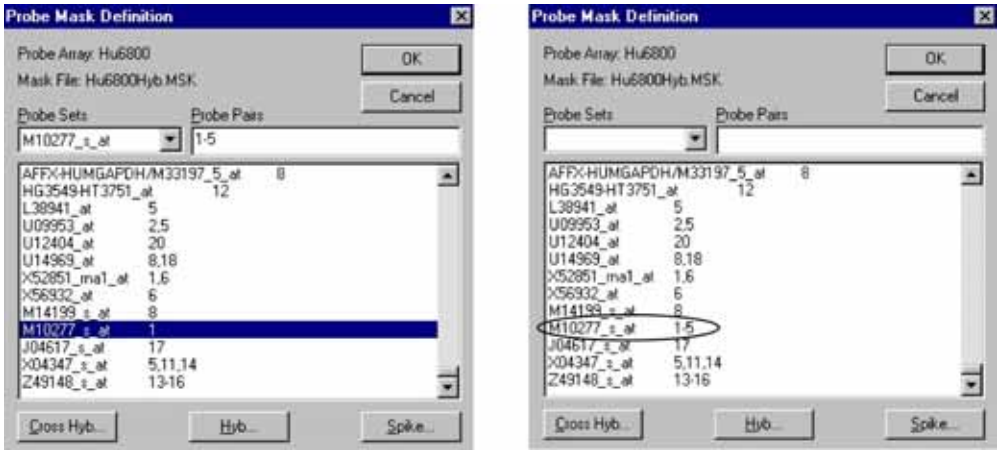


Figure G.31
Edit the entry in the Probe Pairs box (left) and click the lower box to update the probe pair list (right)

4. Edit or delete the entry in the **Probe Pairs** box, then click the lower box to update the list of probe pairs **(Figure G.31)**.

5. Click **OK** when finished to close the Probe Mask Definition dialog box.

Combining Hybridization Probe Masks

A hybridization probe mask may be combined with (*And* or *Or* fashion) or overwrite an existing hybridization probe mask.

1. In the Probe Mask tab, click **Create/Open Probe Mask** **(Figure G.32)**.

   ⇒ The Probe Mask File dialog box appears **(Figure G.33)**.

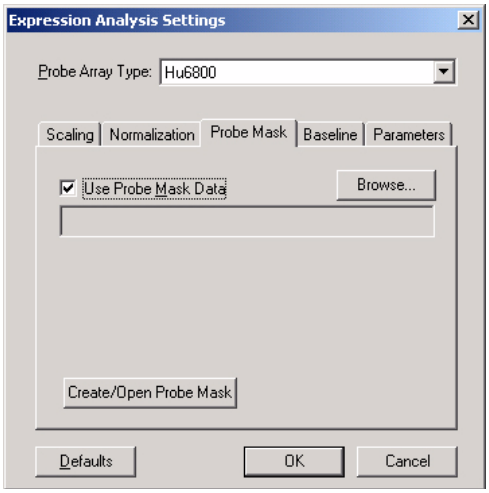


Figure G.32
Expression Analysis Settings dialog box, Probe Mask tab

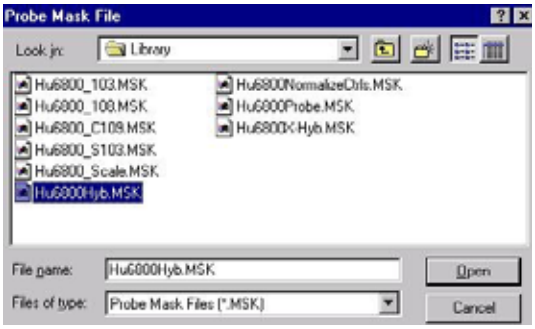


Figure G.33
Probe Mask File dialog box

2. Double-click the hybridization probe mask (.msk) of interest.

   ⇒ The Probe Mask Definition dialog box appears **(Figure G.34)**.

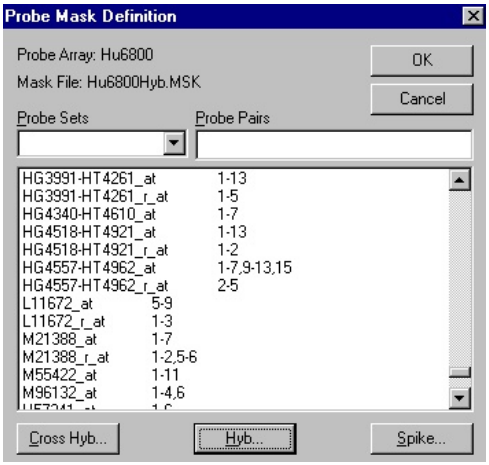


Figure G.34
Probe Mask Definition dialog box

3. Click **Hyb.**

   ⇒ The Hybridization Behavior dialog box appears **(Figure G.35)**.

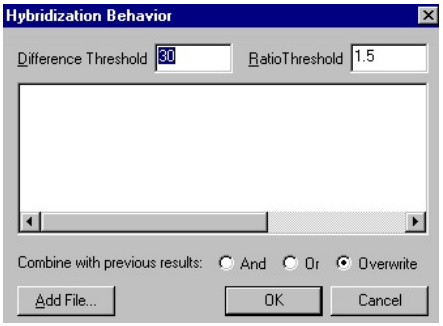


Figure G.35
Hybridization Behavior dialog box

4. Enter new Difference Threshold or Ratio Threshold values if desired.
5. Click **Add File** in the Hybridization Behavior dialog box.
   ⇒ This displays the Open dialog box **(Figure G.36)**.

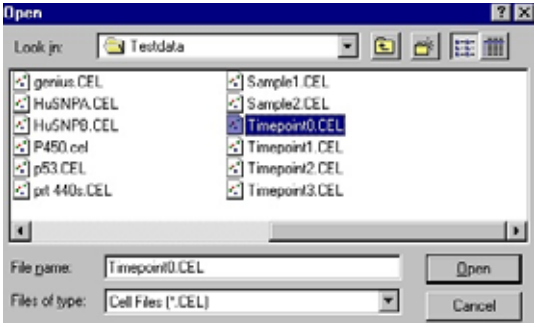


Figure G.36
Open dialog box

6. Double-click the .cel file you wish to combine with the hybridization mask selected in step 2.
   ⇒ The Hybridization Behavior dialog box displays the selected .cel file **(Figure G.37)**.
7. Click **OK**. Repeat step 5 and step 6 to add additional .cel files to the Hybridization Behavior dialog box **(Figure G.37)**.

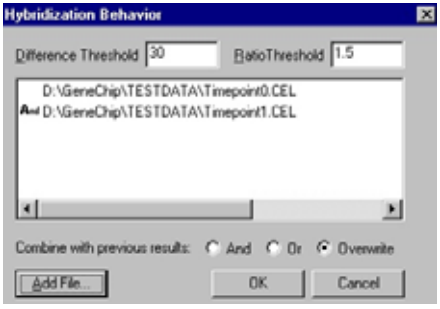


Figure G.37
Hybridization Behavior dialog box

8. Double-click the second (and any subsequent) .cel file name to combine the .cel files in an *And* and *Or* fashion.

9. Choose a **Combine with previous results** option (*And*, *Or*, or *Overwrite*), then click **OK**.

   GCOS analyzes the selected .cel files to identify the probe pairs where PM-MM ≤ Difference Threshold or PM/MM ≤ Ratio Threshold. The software combines the results from each .cel file in an *And* or *Or* fashion to create a new composite Hybridization probe mask.

   This combines the composite hybridization mask with the mask selected in step 2 (for example, Hu6800Hyb.msk in **Figure G.33**) as specified in step 9 (*And* or *Or* fashion, or overwrites the selected hybridization mask).

Spike Probe Mask

GCOS compares two .cel files, one derived from a *spiked* target that contained known amounts of a control transcript(s) and one derived from an unspiked target.

The software identifies any probe pairs where:

$$(PM - MM)_{spike} - (PM - MM)_{unspike} < \text{Difference Threshold},$$

or

$$(PM - MM)_{spike} / (PM - MM)_{unspike} < 1 + \text{Ratio Threshold}$$

and automatically generates a spike probe mask (.msk) comprised of all probe pairs that meet either of the above criteria. The Difference and Ratio Thresholds are user modifiable with defaults of 30 and 1 respectively.

Creating a Spike Probe Mask

1. In the Probe Mask tab, click **Create/Open Probe Mask** **(Figure G.38)**.

   ⇒ The Probe Mask File dialog box appears **(Figure G.39)**.

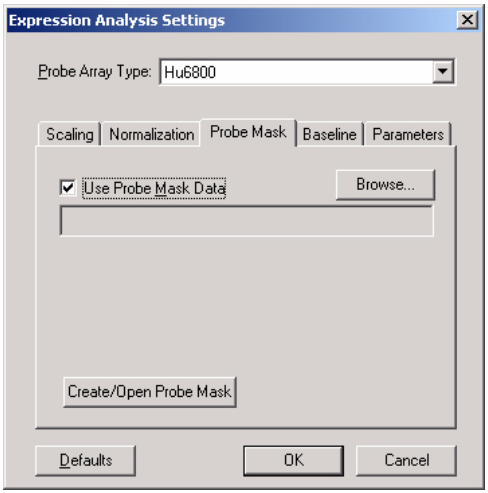


Figure G.38
Expression Analysis Settings dialog box, Probe Mask tab

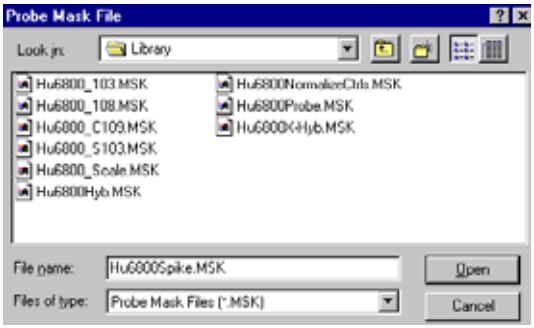


Figure G.39
Probe Mask File dialog box

2. Enter a name in the **File Name** box for the new spike probe mask, then click **Open** **(Figure G.39)**.

   ⇒ The Probe Mask Definition dialog box appears **(Figure G.40)**.

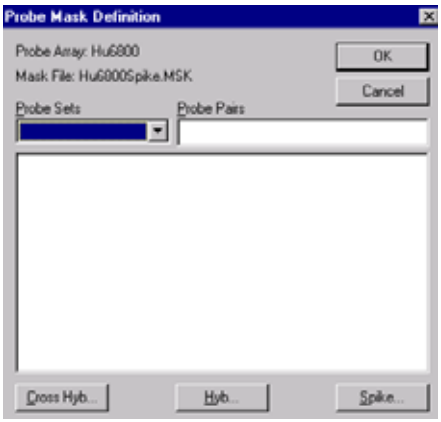


Figure G.40
Probe Mask Definition dialog box

3. Click **Spike.**

   ⇒ The Spike Behavior dialog box appears **(Figure G.41)**.

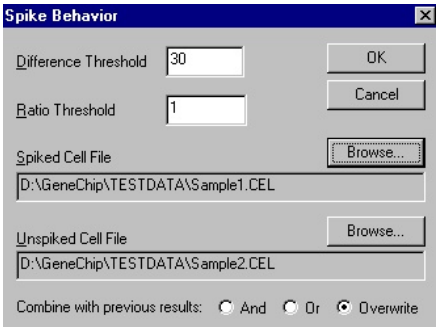


Figure G.41
Spike Behavior dialog box

4. To select a spiked .cel file, click **Browse** (upper button).

   ⇒ This displays the Open dialog box **(Figure G.42)**.

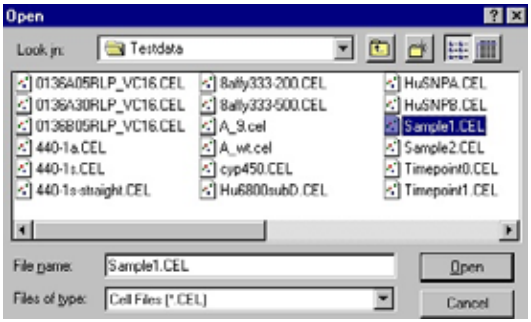


Figure G.42
Open dialog box

5. Double-click the .cel file derived from the spiked target.

   ⇒ This adds the selected .cel to the **Spiked Cell File** box **(Figure G.41)**.

6. To select an unspiked .cel, click **Browse** (lower button) in the Spike Behavior dialog box **(Figure G.41)**.

   ⇒ This displays the Open dialog box **(Figure G.42)**.

7. Double-click the .cel file derived from the unspiked target.

   ⇒ This adds the selected .cel to the **Unspiked Cell File** box **(Figure G.41)**.

8. Click OK to generate the spike probe mask.

   ⇒ The Probe Mask Definition dialog box displays the probe pairs of the spike probe mask **(Figure G.43)**.

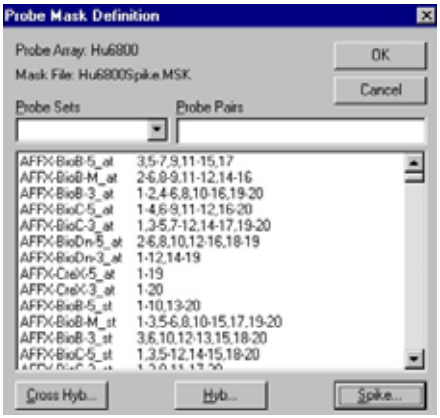


Figure G.43
Probe Mask Definition dialog box

Editing a Spike Probe Mask

1. In the Probe Mask tab, click **Create/Open Probe Mask** **(Figure G.44)**.

   ⇒ The Probe Mask File dialog box appears **(Figure G.45)**.

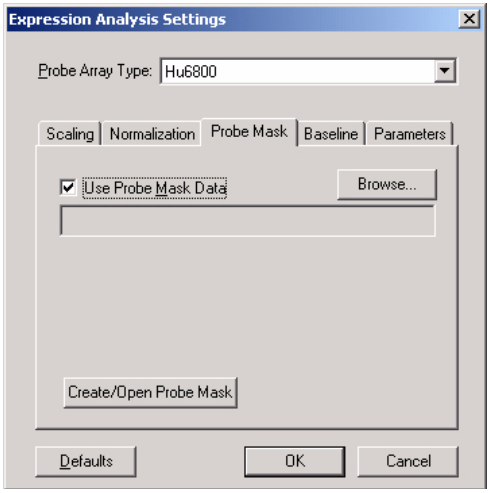


Figure G.44
Expression Analysis Settings, Probe Mask tab

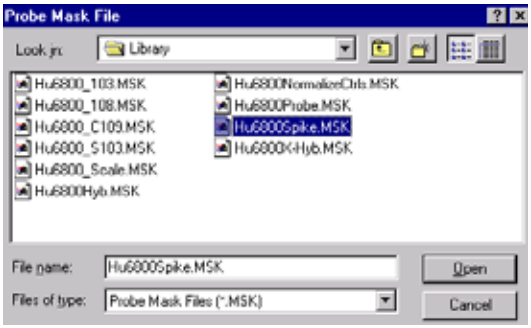


Figure G.45
Probe Mask File dialog box

2. Double-click the spike probe mask you wish to edit **(Figure G.45)**.

   ⇒ The Probe Mask Definition dialog box displays the probe pairs in the selected spike probe mask **(Figure G.46)**.

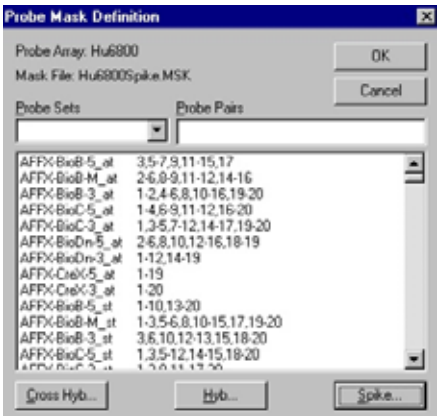


Figure G.46
Probe Mask Definition dialog box

3. In the lower box, click the probe set/probe pair entry you wish to edit.

   ⇒ The Probe Sets box and Probe Pairs box automatically display the selection **(Figure G.47)**.

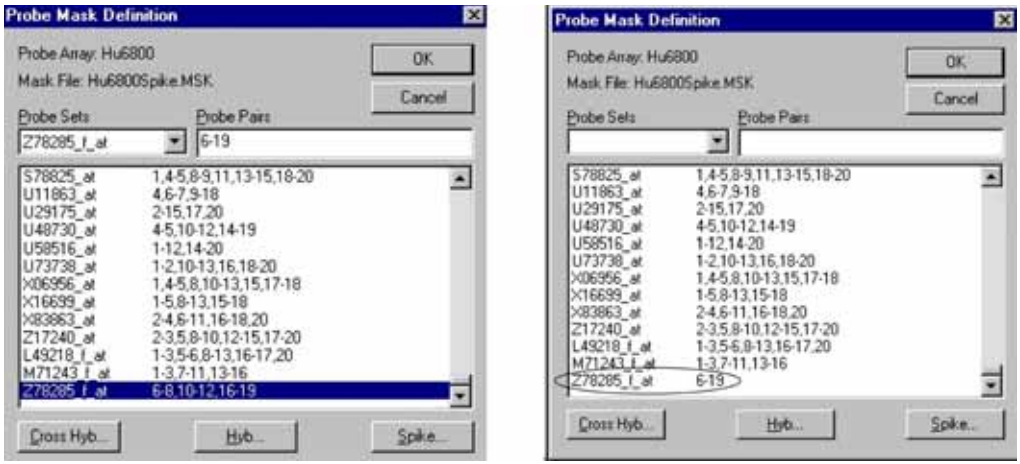


Figure G.47
Edit the Probe Pairs box and click the lower box (left) to update the probe pair list (right)

4. Edit or delete the entry in the **Probe Pairs** box, then click the lower field to update the list of probe pairs **(Figure G.47)**.
5. Click **OK** when finished to close the Probe Mask Definition dialog box.

Combining Spike Probe Masks

A spike probe mask may be combined with (*And* or *Or* fashion) or overwrite an existing spike probe mask.

1. In the Probe Mask tab, click **Create/Open Probe Mask** **(Figure G.48)**.
   ⇒ The Probe Mask File dialog box appears **(Figure G.49)**.

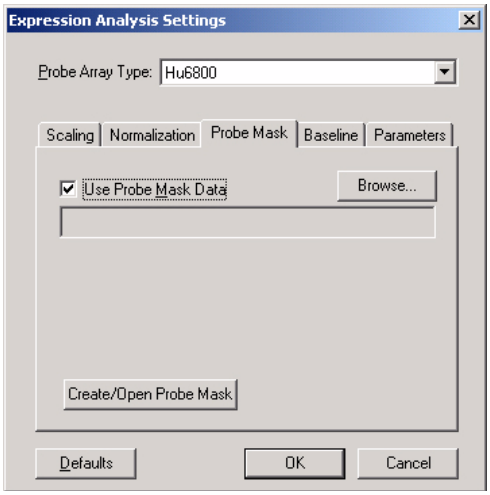


Figure G.48
Expression Analysis Settings, Probe Mask tab

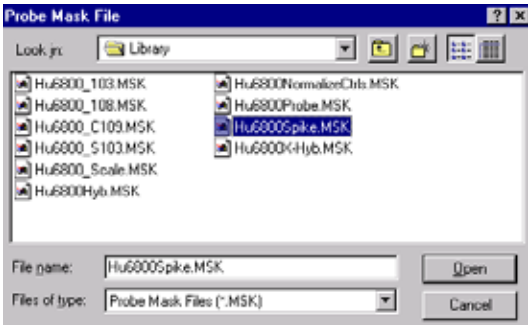


Figure G.49
Probe Mask File dialog box

2. Double-click the spike probe mask of interest **(Figure G.49)**.

   ⇒ The Probe Mask Definition dialog box displays the probe pairs in the selected spike probe mask **(Figure G.50)**.

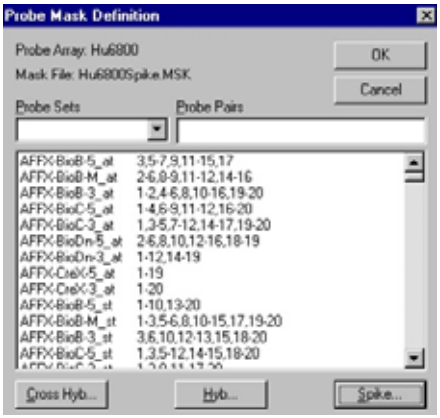


Figure G.50
Probe Mask Definition dialog box

3. Click **Spike.**

   ⇒ The Spike Behavior dialog box appears **(Figure G.51)**.

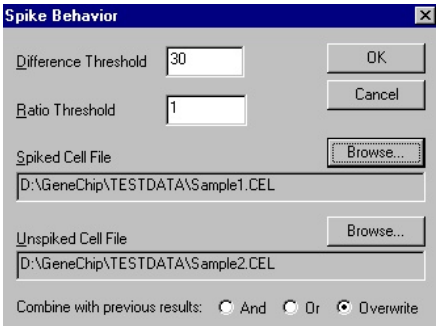


Figure G.51
Spike Behavior dialog box

4. To select a spiked .cel file, click **Browse** (upper button).

   ⇒ This displays the Open dialog box **(Figure G.52)**.

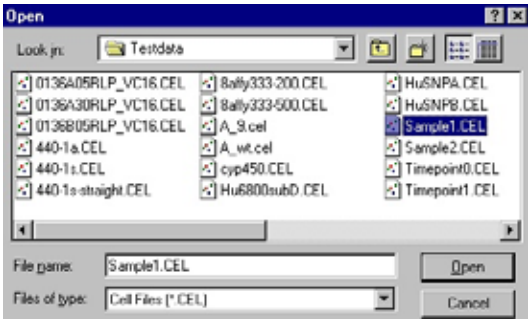


Figure G.52
Open dialog box

5. Double-click the .cel file derived from the spiked target.

   ⇒ This adds the selected .cel to the **Spiked Cell File** box **(Figure G.51)**.

6. To select an unspiked .cel, click **Browse** (lower button) in the Spike Behavior dialog box **(Figure G.51)**.

   ⇒ This displays the Open dialog box **(Figure G.52)**.

7. Double-click the .cel file derived from the unspiked target.

   ⇒ This adds the selected .cel to the **Unspiked Cell File** box **(Figure G.51)**.

8. Select a **Combine with previous results** option (*And*, *Or*, or Overwrite) and click **OK**.

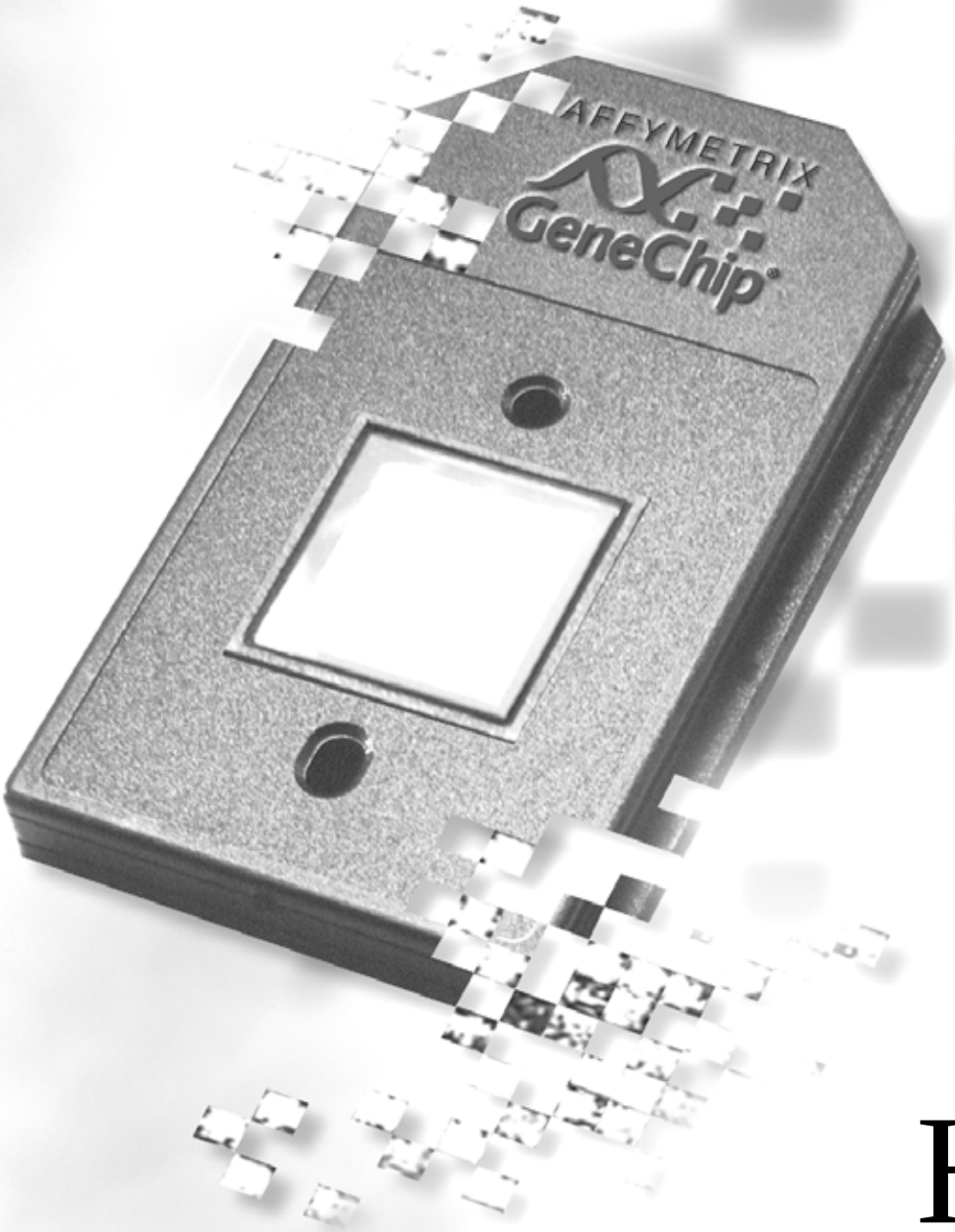
   GCOS analyzes the two selected .cel files and identifies all probe pairs that meet either of the following criteria:

   $$(PM - MM)_{spike} - (PM - MM)_{unspike} < \text{Difference Threshold}$$

   or

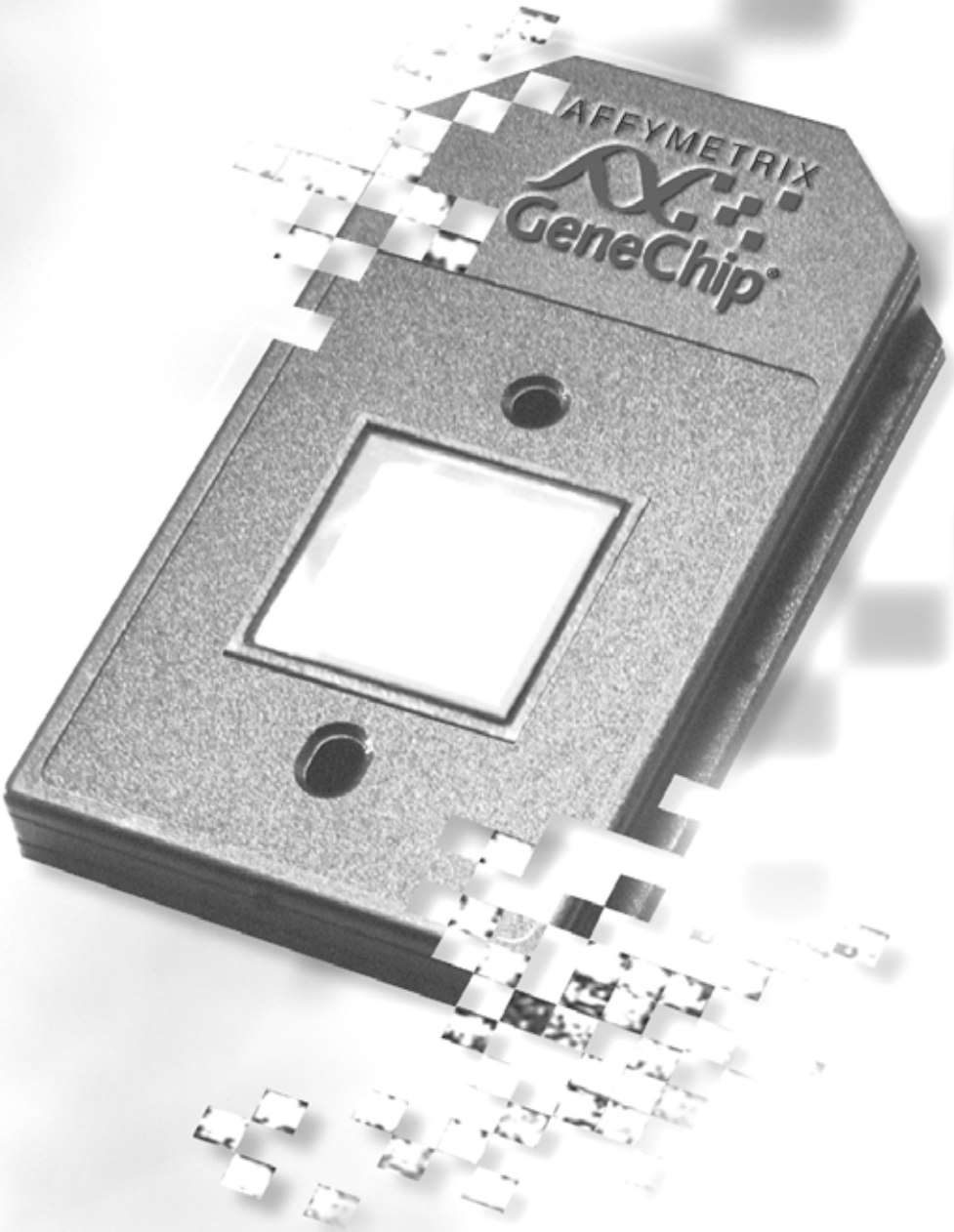
   $$(PM - MM)_{spike} / (PM - MM)_{unspike} < 1 + \text{Ratio Threshold}$$

   The software combines the results from the .cel files to create a new composite spike probe mask. This combines the composite spike probe mask with the spike probe mask selected in step 2 (for example, Hu6800Spike.MSK in **Figure G.45**) as specified in step 8 (*And* or *Or* fashion, or overwrites the selected spike probe mask).



H

Appendix H



Base Codes and Amino Acid Abbreviations

IUPAC Base Codes

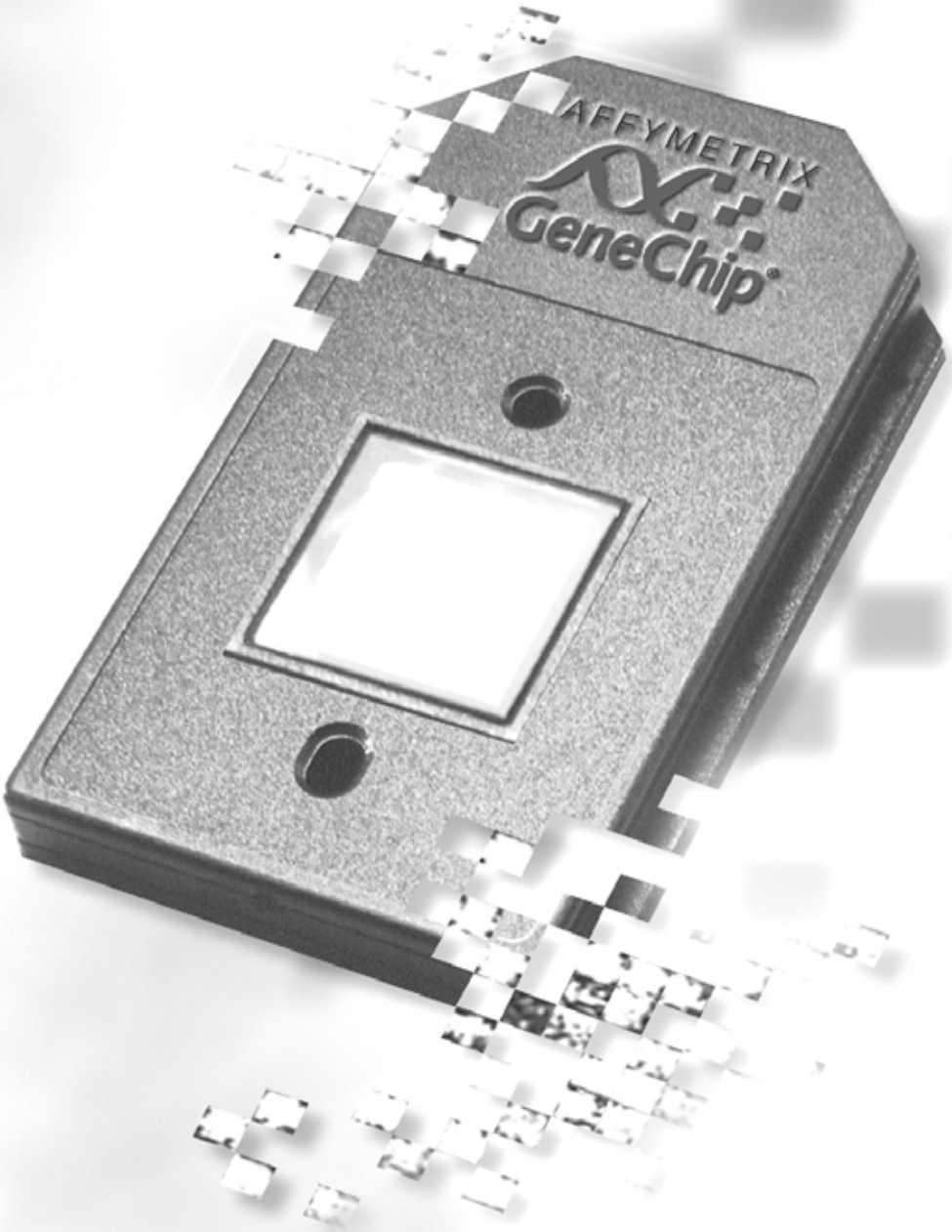
Table H.1
Base Codes

IUPAC Code	Group	Base(s)
A	A	Adenine
C	C	Cytosine
G	G	Guanine
T	T	Thymine
M	A or C	aMino
R	A or G	puRine
W	A or T (U)	Weak interaction (2 H bonds)
Y	C or T (U)	pYrimidine
S	C or G	Strong interaction (3 H bonds)
K	G or T(U)	Keto
V	A or C or G	not-T or not-U (since V follows U)
H	A or C or T(U)	not-G (since H follows G)
D	A or G or T(U)	not-C (since D follows C)
B	C or G or T(U)	not-A (since B follows A)
N	A, C, G or T(U)	aNy

Amino Acid Abbreviations

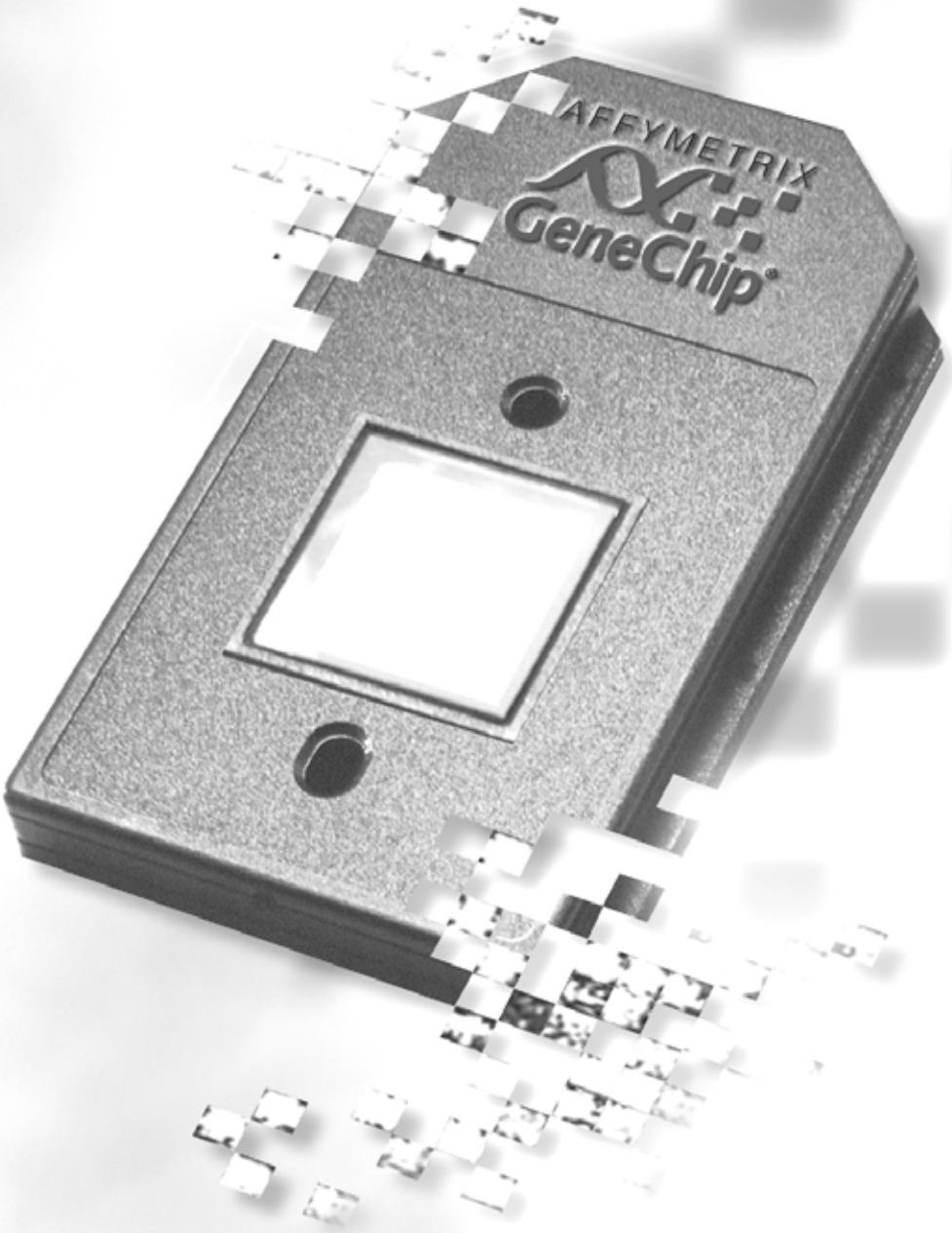
Table H.2
Amino acid abbreviations

One Letter	Three Letter	Amino Acid
A	Ala	Alanine
B	Asx	Asparagine or Aspartic acid
C	Cys	Cysteine
D	Asp	Aspartic acid
E	Glu	Glutamic acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	Hi	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
Y	Tyr	Tyrosine
Z	Glx	Glutamine or Glutamic acid



I

Appendix I



Toolbars, Hot Keys, & Windowpanes

This appendix reviews toolbar button functions, hot keys, and explains how to resize windowpanes or columns.

Toolbars

You can display toolbars with text labels **(Figure I.1)**. To display the toolbar button labels, select **View →Toolbar →Text Labels** from the menu bar.

GCOS Toolbars

Main Toolbar

Figure I.1
Main toolbar

Table I.1
Main toolbar

Menu Bar Command	Toolbar Button	Function
File →Open		Displays the Open dialog box so that data (for example, probe analysis data or image data) may be opened.
File →Save		Saves the open image, experiment, or report.
File →Print		Displays the Print dialog box.

Table I.1
Main toolbar

Menu Bar Command	Toolbar Button	Function
Window ⟶Data Tree		Displays or hides the data tree.
Window ⟶Shortcut Bar		Displays or hides the shortcut bar.
Window ⟶Status Log		Displays or hides the status log.
Run ⟶Experiment Info		Displays experiment window.
Run ⟶Fluidics		Displays the Station Selection dialog box for the Affymetrix® Fluidics Station 400.
Run ⟶Scanner		Displays the Scanner dialog box.
Run ⟶Stop Scanner		Stops a scan in progress.
Run ⟶Analysis		Runs an analysis on the open *.dat or *.cel data.
Edit ⟶Image Settings		Displays the Image Settings dialog box.
Help ⟶Contents		Displays GCOS help.

Expression Analysis Window (EAW) Toolbar

Figure I.2
EAW toolbar

Table I.2
EAW toolbar button functions

Menu Bar Command	EAW Toolbar Button	Function
Edit →Find		Displays the Find Probe Set dialog box.
Edit →Sort		Displays the Sort dialog box.
View →Hide Selected		Hides selected probe set(s) in the metrics or pivot table.
View →Hide Unselected		Hides unselected probe set(s) in the metrics or pivot table.
View →Unhide All		Displays all probe sets previously hidden in the metrics or pivot table.
Analysis →Options		Displays the Analysis Options dialog box.
Graph →Clear Selected Graphs		Clears selected graphs from the graph pane in the EAW.
Graph →Scatter Correlation Graph		Displays the Scatter Graph dialog box.
Graph →Series Graph		Displays the Select Series Graph Parameter dialog box.
Graph →Intensity Bar Graph		Plots the Intensity Bar Graph for the selected probe set(s).
Graph →Measured Images		Displays the hybridization intensity image data (.dat) for the selected probe set(s).

Table I.2
EAW toolbar button functions

Menu Bar Command	EAW Toolbar Button	Function
Graph ⟶Lasso Points		Changes the cursor to a drawing tool that enables the user to draw a circle around points in a scatter graph.

Batch Analysis Toolbar

Figure I.3
Batch analysis toolbar

Table I.3
Batch analysis toolbar button functions

Menu Bar Command	Batch Analysis Toolbar Button	Function
Edit ⟶Add Item		Displays the Open dialog box to select and add *.cel files to the Batch Analysis window.
Edit ⟶Remove Item		Removes the selected *.cel files from the Batch Analysis window.
Edit ⟶Start Analysis		Starts the batch analysis.
Edit ⟶Stop Analysis		Stops the batch analysis.
View ⟶Options		Displays the Batch Analysis Options dialog box.

Report Toolbar

Figure I.4
Report window toolbar

Table I.4
Report toolbar button functions

Menu Bar Command	Report Toolbar Button	Function
Edit →Cut		Removes a highlighted selection from the report.
Edit →Copy		Copies a highlighted selection in the report to the system clipboard.
Edit →Paste		Pastes a copied or cut selection at the current insertion point.
Edit →Find		Displays the Find dialog box.
Edit →Find Next		Performs a text search for the item specified in the Find dialog box.

Publish Toolbar

Figure I.5
Publish window toolbar

Table I.5
Publish toolbar button functions

Menu Bar Command	Publish Window Toolbar Button	Function
Publish →Add Item		Displays the Open dialog box to select and add experiment data (*task items*) to the publish task.
Publish →Remove Item		Removes the selected task item(s) from the publish task.
Publish →Publish		Send the task to the GCOS server to be published during the time specified by the GCOS Server administrator.
Publish →Monitor → Cancel Publish		Cancels a task (changes the task status from WAIT to CANCELED).
Publish →Monitor → Restart Publish		Reinstates a canceled task to be published during the time specified by the GCOS Server administrator (changes the task status from CANCELED to WAIT).

GCOS Manager Toolbar

Figure I.6
GCOS Manager toolbar

Table I.6
GCOS Manager toolbar button functions

Menu Bar Command	GCOS Manager Toolbar Button	Function
Process →Print Experiment		Prints experiment information.
Tools →Register GCOS Server		Opens the Register GCOS Server dialog box
Tools →Server Event Viewer		Opens the Server Event Viewer
Tools →Server Tasks		Displays the Task List for the active server.
Tools →File Path		Displays the Select Export File Path dialog box
Tools →Find		Opens the Find dialog box
Tools →Filter		Opens the Sample Filters dialog box
Tools →Help Topics		Opens GCOS Manager online help.

GCOS Administrator Toolbar & Icons

Figure I.7
GCOS Administrator toolbar

Table I.7
GCOS Administrator toolbar button functions

Menu Bar Command	GCOS Administrator Toolbar Button	Function
		Opens the Administrator Password dialog box so that a system administrator password or publish database password can be created and updated.
		Refreshes the data tree in the copy or space management view. Refresh the data tree to view new data or databases added to the system during the current session.
Tools →Register GCOS Server		Opens the Register GCOS Server box.
Tools →Server Tasks		Opens the Sample Filters dialog box.
Tools →Help Topics		Opens GCOS Manager online help.

Table I.8
GCOS Administrator Icons

	Process Database or LIMS System Database (yellow)		Project		Sample
	Publish Database (gray)		Experiment Information		Filter

Hot Keys

Table I.9
GCOS hot key descriptions

Menu Bar Command	Hot Key
File →Open	Ctrl + O
File →Print	Ctrl + P
Edit →Copy	Ctrl + C
File →Save	Ctrl + S
Edit →Image Settings	S
Edit →Delete	Del
Edit →Find Next	F3
Edit →Unmask All Cells	Shift + U
View →Grid	G
View →Probe Cell Data	C
View →Corner →Upper Left	F5
View →Corner →Upper Right	F6
View →Corner →Lower Left	F7
View →Corner →Lower Right	F8
View →Image →Measured	M
View →Image →Difference	D
View →Image →Average	A
View →Next Highlight	N
View →Previous Highlight	P
View →Clear Highlights	E
View →Probe Tiling	T

Table I.9
GCOS hot key descriptions

Menu Bar Command	Hot Key
	1, 2, 3, etc. (toggle between open image windows)
	I (Image window zoom in)
	O (Image window zoom out)
	Shift+O (Image window full zoom out)
	L (intensity autoscale)
Edit →Mask Cells	X
Edit →Unmask Cells	U
Help	F1
Scroll up one page	Page Up
Scroll down one page	Page Down
Scroll left one page	Ctrl + Page Up
Scroll right one page	Ctrl + Page Down
Scroll up 1/10 page	Up arrow
Scroll down 1/10 page	Down arrow
Scroll left 1/10 page	Left arrow
Scroll right 1/10 page	Right arrow

Table I.10
GCOS Manager hot key descriptions

Menu Bar Command	Hot Key
View → Refresh	F5

Working with Windowpanes & Columns

You can resize windowpanes.

1. Place the mouse pointer over a windowpane border so that it changes to a double arrow ↕ **(Figure I.8)**.

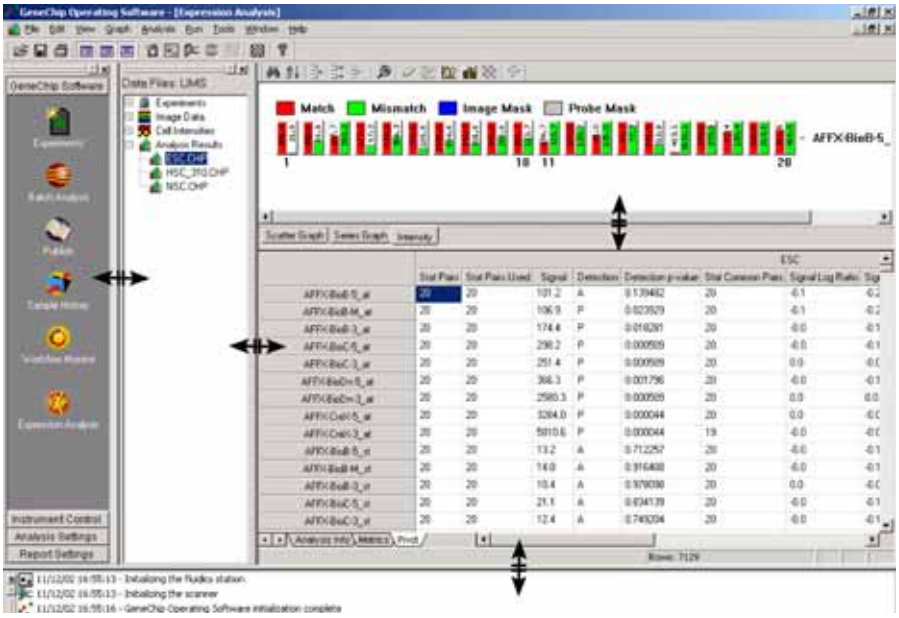


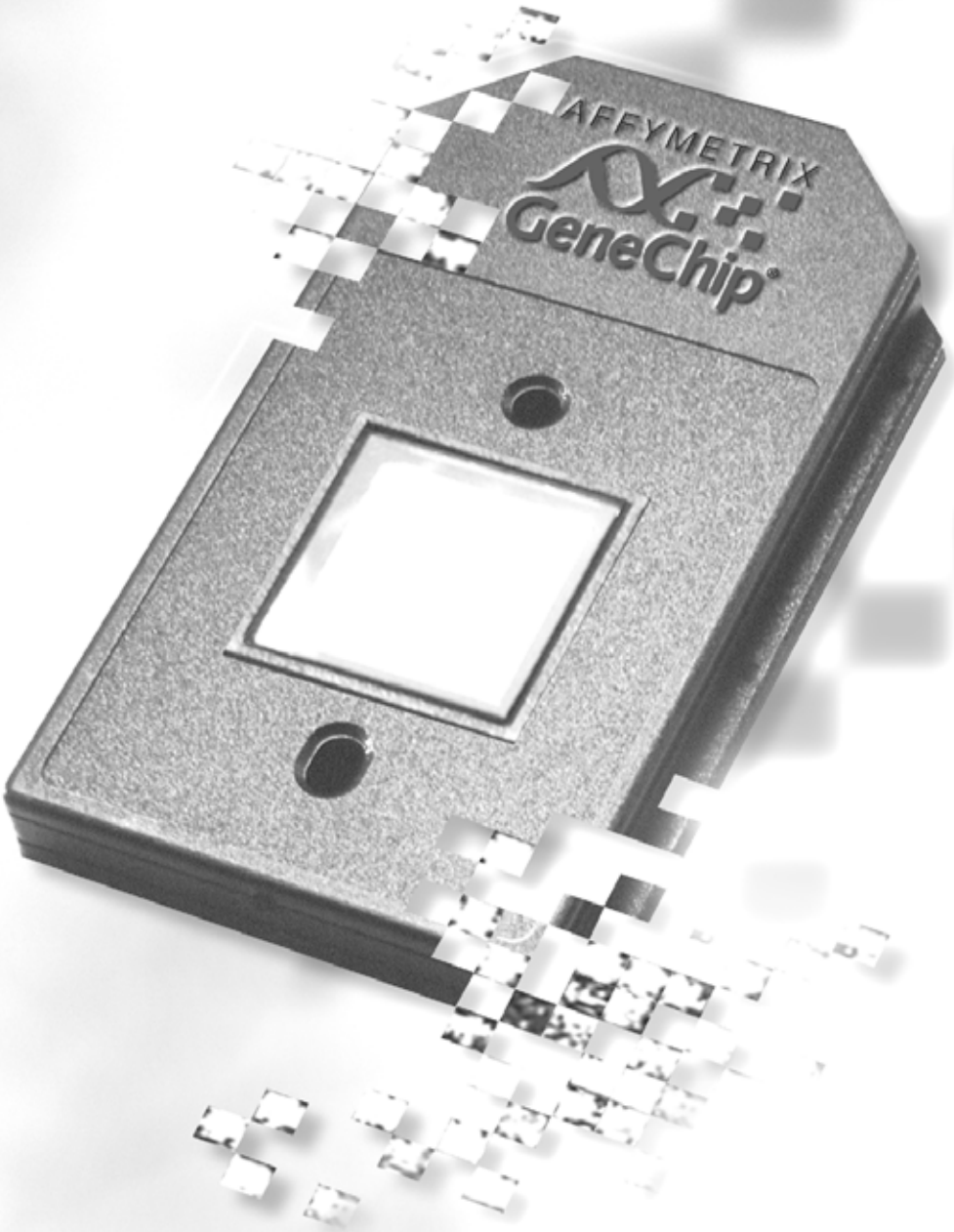
Figure I.8
Resize windowpanes horizontally or vertically

2. Drag the border to resize the windowpane.

Resizing or Hiding Columns in the EAW

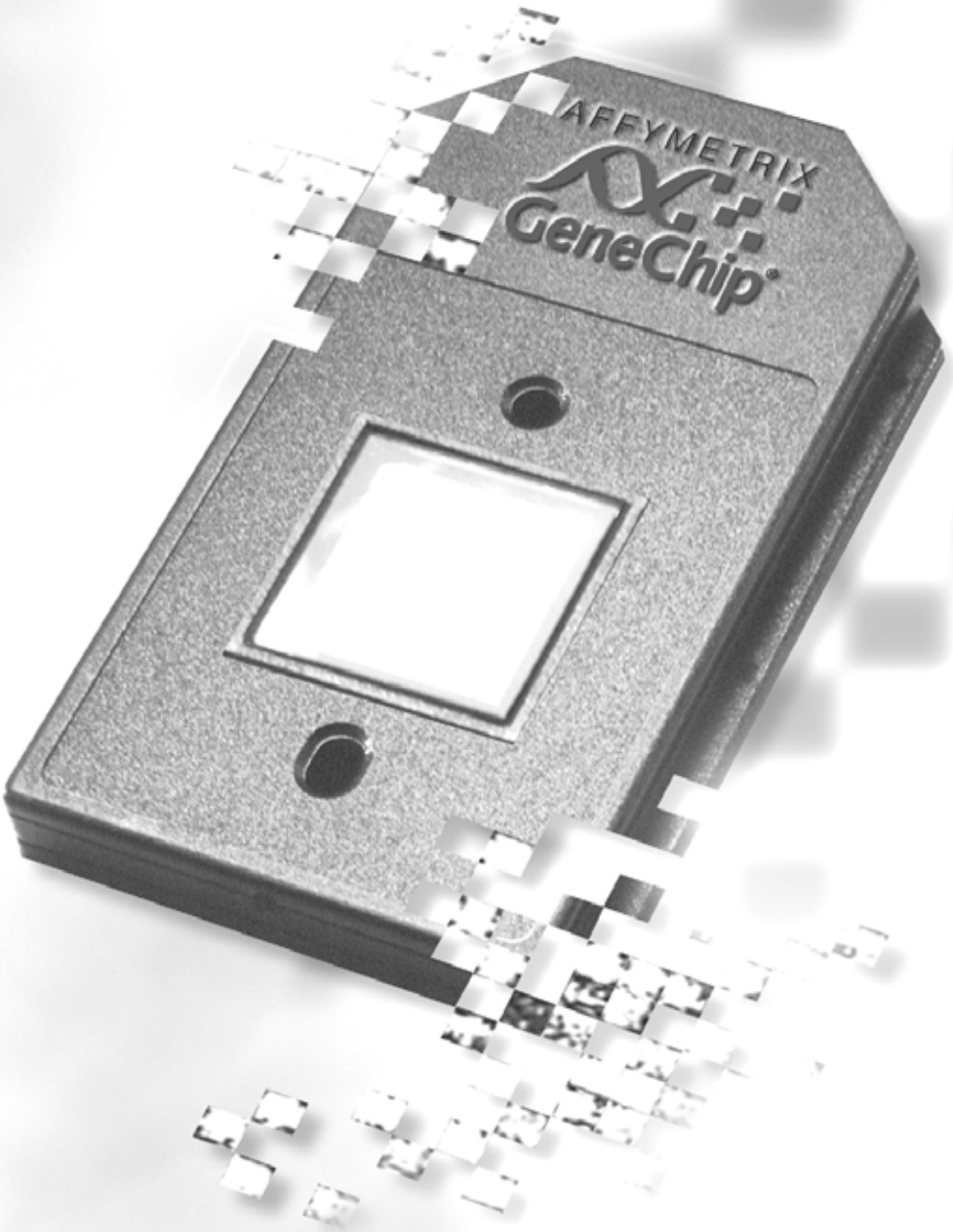
You can resize or hide columns in the Expression Analysis window (EAW). GCOS stores the column settings for future sessions on a per user basis (identified by the logon name) so that one user's settings do not affect the settings of another.

1. Position the mouse over the left or right cell border in the column header so that it changes to a double arrow ↔ .
2. Drag the cell border to resize the width of the column.
3. To hide a column, drag the left or right cell border of the column header until the column width is reduced to zero. Alternatively, right-click the column header in the EAW and select **Hide Column** from the shortcut menu that appears.



J

Appendix J



Database Management & Data Source Name Descriptions

Performance Tuning for SQL Server Databases

Microsoft® SQL Server automatically tunes many of the server configuration options, therefore requiring little, if any, tuning by a database administrator. Although these configuration options can be modified by the database administrator, it is generally recommended that they are left at their default values, allowing SQL Server to automatically tune itself based on run-time conditions.

However, if necessary, the following components can be configured to optimize server performance:

- SQL Server Memory
- I/O subsystem
- Microsoft® Windows options

To improve performance of querying, it is recommended that the SQL Server database is in three devices: data, index and log. With this separation, reading and writing of data is improved which helps decrease amount of time it takes to query data.

Data Device

The data device stores the experimental data, published data, and other data associated with GCOS. These data are accessed by GCOS and all applications that work on GCOS. The name of this device is <*publish database name*>_DAT.mdf.

✔ NOTE *To move the locations of data devices from one disk to another, use the Space Manager tool in GCOS Administrator.*

To improve performance, the Data Device file should be placed on a dedicated physical disk.

Index Device

The Index Device is available for publish databases. The Index Device provides quick access to data and can enforce uniqueness on the rows in a table. With this device, it allows searches in a database program to find data in a table without scanning the entire table. This device is a list of values in a table with the storage locations of rows in the table that contain a value. The name of this device is
<*publish database name*>_INDEX_mdf.

✔ NOTE *To move the locations of data devices from one disk to another, use the Space Manager tool in GCOS Administrator.*

Basic Configuration

The index files can be located with the Data Device files, however, performance may be gained by placing the Index Device file on a different disk with low activity.

Advanced Configuration

Place the Index Device file on a dedicated physical disk. If a remote AADM server is used, place the Data Device file on a dedicated physical disk.

Log Device

The Log Device holds the information used to recover the database. There must be at least one log file for each database.

✔ NOTE

To move the locations of log devices from one disk to another, use the Space Manager tool in GCOS Administrator.

Basic Configuration

The Log Device file can be placed where there is space.

Advanced Configuration

Place the Log Device file on a dedicated physical disk.

If a remote AADM server is used, place the Data Device file on a dedicated physical disk.

Additional Performance Tuning Information

These are other suggestions that can help improve the performance of your system.

- Separate all devices SQL Server uses. For example, place the data device, index device, and log device each on their own individual physical disk. The separation of these files improves the performance by allowing the drive heads to perform read only or write only operations during the majority of the publishing operation.
- Use a remote AADM server for publishing and place the data device, index device, and log device each on their own individual physical disk.
- Placing both the index device and log device on the same disk can slow performance since there is a lot of read and write activity being performed.

Client Tuning

*As part of the software requirement for the client, it is recommended to install SQL*Loader on the client running Data Mining Tool if connecting to an Oracle® GCOS server. Also, a registry setting can be added on the client machine when preforming a large pivot.*

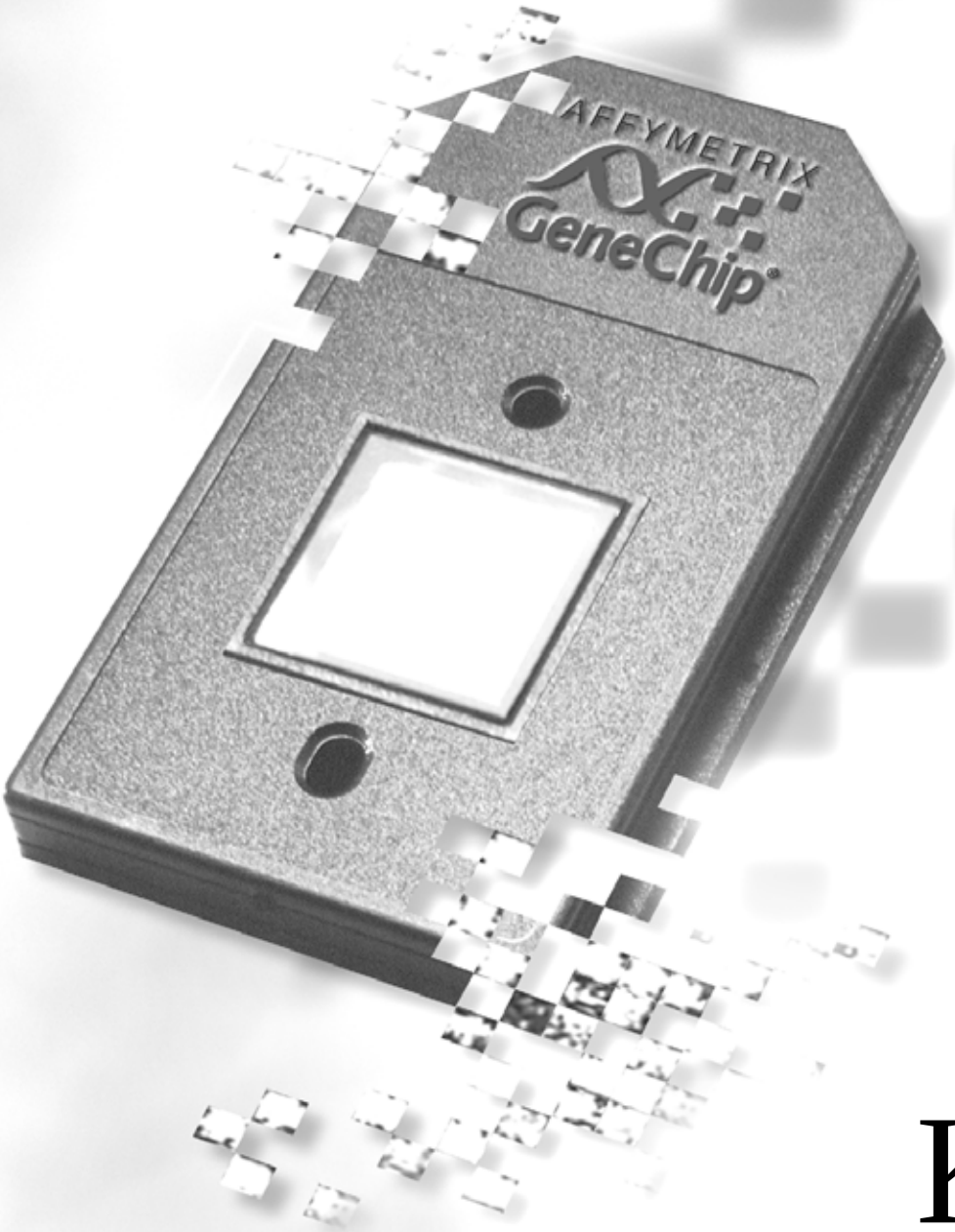
SQL*Loader

If your GCOS server uses an Oracle® database, it is advisable to install SQL*Loader on the client workstation. Contact your Database Administrator to install SQL*Loader. SQL*Loader is included on the Oracle® installation CD.

SQL*Loader is used to bulk loading probe set lists and annotations that users save into the database. With SQL*Loader installed on the client, the process of saving large amounts of data at time is improved. Without SQL*Loader installed on the client, it may take a long time to save the information.

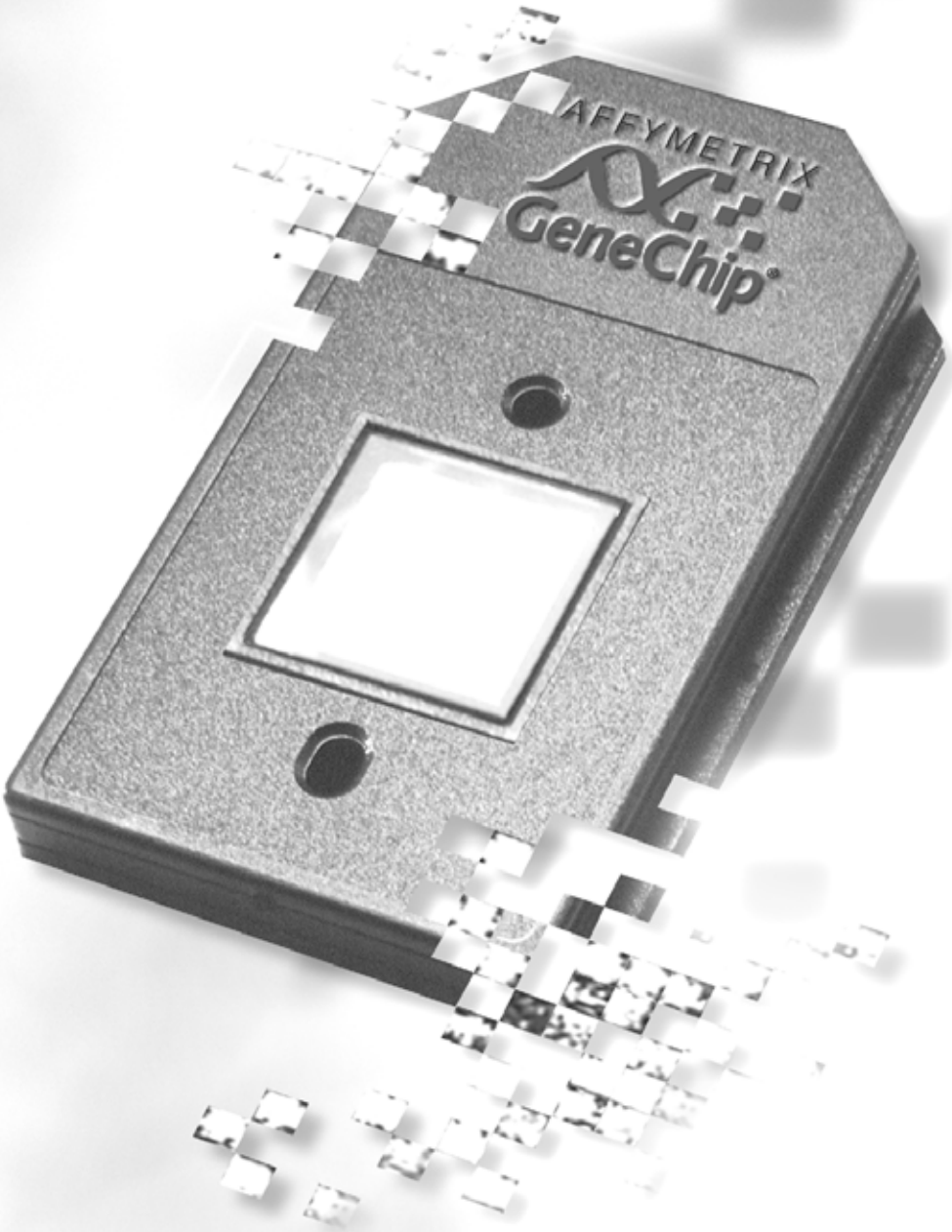
✔ NOTE

*The same version of SQL*Loader must be installed on the workstation as on the GCOS server.*



K

Appendix K



Troubleshooting

This section of the manual helps you troubleshoot problems that may be encountered during installation or administration of the server and the different software products on the clients.

This will also help you with some basic troubleshooting techniques and what information should be gathered to help Technical Support work towards providing a resolution to a potential problem that you are experiencing.

Troubleshooting Questions

Some basic tests may quickly reveal the cause of the problems. It is important to isolate the problem down to a particular area. Some questions to answer before calling Technical Support for help:

Is it a client or a server problem?

If server, has anything changed? Addition of software? Addition of network functions? Restoring of databases?

Check the Event log.

Check the SQL Server log (if using a Microsoft® SQL Server database).

Which database is being accessed?

Is there enough free space available?

Can you reproduce the problem? What steps were taken?

What is the exact error message?

Does it happen with a particular user or all users?

What has changed on the client?

Is the user logged onto the correct domain? Does the user have permissions to access GCOS Server?

Can you write to the publish database?

Can you access other databases?

Can you reproduce the problem? What steps were taken?

What is the exact error message?

GCOS 1.0 Troubleshooting

Installation

- **I am unable to install the software.**

  Verify that you have administrative privileges in order to install the software.
- **Error connecting to GCOS Server database.**

  Check the connectivity. Check the server is running.
- **When installing GCOS 1.0 the installation tries to copy files, however, the error message “cannot move error - 145” appears. What should I do?**

  Verify that all Affymetrix applications are closed and not running. Log in as another user and, again, try installing the application. It may be the user’s profile is corrupted.
- **The GCOS software shows the following dialog box when installing the software or MSDE fails to install.**

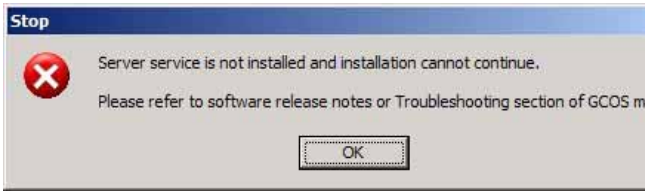


Figure 16.22
Stop message

GCOS requires MSDE. In order to install MSDE, the "Server" service also known as the "lanmanmanger" service needs to be installed and running. GCOS will attempt to start the service if it has stopped.

To see if the "Server" service is installed, right-click "My Computer" and select "Manage". Navigate to the "Services and Applications" Node". Select Services and see if the Server service is installed.

To install the Server service the workstation must have a Network Interface Card (NIC) or a Microsoft loop-back adapter. Once the NIC has been installed, the "Server" service can be installed by adding the "File and Printer Sharing for Microsoft Networks" to the Local Area Connection properties. Refer to www.microsoft.com or contact your system administrator for details on installing the server service.

Installing Pre-GCOS Version Libraries (LIMS 3.0 Libraries) on the Client

In order to install LIMS 3.0 version libraries (pre-June 2003 libraries) on a GCOS client, you must complete the following four steps.

1. Run ToggleLIMS3LibrarySupport.exe file by double-clicking the file. (The ToggleLIMS3LibrarySupport.exe file is located in the directory where GOCS was installed.) This enables the older library installer and temporarily disables GCOS.
2. Install the old version library.
3. Run the ToggleLIMS3LibrarySupport.exe file by double-clicking the file again. This enables GCOS and disables the old library installer. GCOS cannot be run until this step is completed.
4. Run the library migrate program (from the subdirectory 'Migrate' contained in the GCOS Target directory, or a new version library install CD).

Publishing

- **Unable to publish data. The server task window displays that it is in the queue and says "wait".**

  Verify that the publishing service is active in the server task window of GCOS 1.0. If the service is stopped, contact the system administrator to start the gcdoservice.
- GCOS shows "Publishing Stopped" when a publish window is launched.

  Check if CGCDOService is started.

- **Unable to publish the .chp file. The user is able to publish any .exp and .cel files, however, when publishing the .chp file, the error message "Failed to publish x.chp: SQL Command Failure."**

  This is possibly due to a probe array type that has been installed and published. However, a new probe array with the same name containing new probe information was installed. The probe set names were changed and therefore do not match and that is why the .chp file with the new probe array information cannot be published.

  Workaround: Delete the data that is published from that probe array type. Reanalyze that existing .chp files and publish again.

- **Unable to Publish to an Oracle® GCOS Server system and the Oracle® Publish database was created successfully.**

  Check the registry setting: HKLM\Software\ODBC\ODBC.INI on the GCOS server.

  Locate the Oracle ODBC DSN created <servername>_ORCL_GATC.

  Verify the values:

	Oracle® 8.1.7
String Value Name	**Value**
HOST_OS	WinNT4
HOST_SQLLOAD	sqlldr

Note: String values are case-sensitive

For the Publish database: <publish database name>_AffyPub:

String Value Name	**Value**
PWD	<encrypted password>
UID	<publish database name>
Alias	<name of the alias>

Note: The name of the alias created points to the GATC publish server

Do the temporary files get created? *.ctl on the root of the c: drive for the Oracle database.

The temporary files on the c:\ drive for SQL Server are s*.* (short file names).

There must be enough free space to create these temporary files (at least 400MB).

- **"Failed to read <name of experiment> from GCOS Server for the .dat file" message appears when publishing.**

There is no .dat file. The experiment needs to be scanned and is currently in the scan stage.

- **"Failed to read <name of experiment> from GCOS Server for Chip." message appears when publishing.**

  There is no object file. The experiment is currently in the hybridization queue and needs to be hybridized and scanned.

- Publish Task Queue should be purged from time to time. As the task queue grows, especially above 10,000 entries, GCOS will appear to hang temporarily when placing jobs in the publish queue. You may want to backup the text of this log prior to purging.

Licensing

- **Upon Launching GCOS 1.0, the license window appears. I enter the license, however the window keeps appearing.**

  Verify that when entering the license, the license number is entered exactly as seen. The license is case sensitive. Ensure that you do not enter any additional spaces.

Workflow

- **Failure in GetSampleBy name command error.**

  Verify that you did not enter any illegal characters used when creating a sample or experiment. Some illegal characters include: < >:;\" ^|*/?

- **Cannot connect to Affymetrix GCOS Server.**

  Verify that the network is up. Also, verify that the server is online. On the client side, verify that the registry key values are correct.

- **Experiments don't show up in the data tree.**

  Verify your filter is not filtering out everything, especially the date range.

- **When trying to reanalyze an analysis after an upgrade in GCOS, the error message "Base call failed for \\<servername>\gcgcosserver\data\\<file.chp> - Invalid base call algorithm. Verify base call default settings for this Probe Array Type or verify Tools\Defaults\File locations settings" appears.**

  One indication of this error message is that the library file update on the server may not have updated properly.

GCOS Manager 1.0 Troubleshooting

Installation

- **When trying to register a GCOS server or click a tab in GCOS Manager, the error message “GCOS Server Component not installed” appears. What does this mean?**

  This means that there is a permission or access problem, or may occur where the shared pool size is too small.

  Verify that the user has permissions to access the server. The logon name is part of the GCOS Server Global Group.

  Verify that the user is registering a server with the GCOS Server components installed.

  Verify that the database services are started (either SQL or Oracle).

- **The Roles tab in GCOS Manager is missing.**

  The Roles tab is unavailable when you work with GCOS connected to the local MSDE database server.

  The global group may have the wrong domain name prefix in the GCOS Server Admin database table 'USERS'. This will happen if your MIS changes the name of the domain. As users begin to migrate to Win2K from NT, some administrators might change the domain name. If they do, you will need to change the domain name in the 'USERS' table.

- **Slow connection when trying to connect to the server with one of the client applications (i.e.: GCOS Manager). What is going on?**

  If users experience slow connection to the server, verify that the Domain Controller and DNS are properly configured and registered with other Domain Controllers within the network.

Delete

- **Unable to delete data from the process database.**

  Verify the error message. Error message displayed “Failed to read experiment: xxx.EXP” It is possible that the data was migrated in from a beta version and the character limit exceeded 25 characters. In this case, the entire sample must be deleted in order to remove the file in question.

- **When trying to delete data from a Publish database, it displays “failed” When viewing the log, the message “IpublishData – Failure**

in CheckForOpenConnections: cannot delete database with open connections (2)" What does this mean?

If another user has the database open (using DMT or publishing data), data cannot be deleted. This is because the tables are locked and cannot be accessed. Wait until the users are done publishing and not using DMT.

Archiving

- **Unable to archive. Error message "Archive File Copy Error".**

  Verify that the path location specified for archiving has enough free space to archive the file(s) selected.

Importing

- **Unable to import. I get a copy file error message in the text log.**

  Verify that the permissions are set properly.

  There is enough free space to import the files.

  If the data is on a CD, try copying it to the local hard drive and import again.
- **Trying to import and the error message "Cannot modify experiment <name of experiment>. It is waiting to be hybridized, scanned, or it's image file requires grid alignment."**

  The experiment that is selected to be imported cannot be imported because this experiment is already on the queue and is waiting to be completed by one of the above steps.
- **Trying to import data and it fails. Verify that the .exp and .dat files exist with the same name.**

  Verify that the files are a valid size. If the data is on a jaz disk, CD-ROM, or other media, try copying it to the local hard drive and import again.

Usersets

- **In GCOS Manager, the user is unable to create a userset.**

  Verify that the information entered is correct.

  Verify that the userset parameters are valid in GCOS Manager.

  Verify that the library files have been installed or updated.
- **In GCOS Manager, I click the Usersets tab and get the message "Failed to load probe arrays -?|"**

  This means that there have not been any probe arrays (library files) installed.

Templates

- **Unable to create a template.**

  Verify that there is not any other template with the same name. Verify that the template was saved.

Exporting

- **Unable to export. Error message "CopyFile Error=112".**

  Verify that the path location specified for exporting has enough free space to write the .exp, .dat, .cel and .chp files.

Publish

- **Unable to create new Publish database.**

  Verify that the username and password entered are correct.

  Verify that the path location specified exists.

  Verify that the path location specified has enough hard disk space.

  Verify that the open cursors set to 200 (Oracle databases only).

  Verify that the publish database name does not start with a number. All publish databases and database passwords must begin with a letter.

- **Creating a SQL Server Publish database. The error message "Device too small" or "Device too large" is displayed. What should the creation size be?**

  The size of creating the publish database is between 128 MB and 1024 MB.

- **Trying to create an Oracle® publish database and it fails.**

  Verify that the username and password entered is correct. The password for the username gatc is 'gatc' (all lowercase). Verify that the path exists. Verify that the open cursors is set to 200. Verify there is enough hard disk space.

- **Creating an Oracle® database and it fails. The log shows that there are not enough cursors. What does this mean?**

  Verify that the INIT.ORA contains the open_cursors =200 (It needs enough cursors to create a database, otherwise all cursors are locked and in use).

- **Unable to delete Publish data from the publish AADM database.**

  The publish data selected to be deleted may be in use by another user. Other users may have the Data Mining Tool application open and

connected to that publish AADM database. Therefore, in order to delete the data, the other user must exit out of DMT.

- **After removing a publish database from the database application, the database name still appears in the publish tab. Why?**

  The system DSN still exists in the ODBC Control Panel. Remove the DSN and it will not appear in the application.

GCOS Manager FAQs

This section of the manual lists frequently asked questions (FAQs) regarding Affymetrix GCOS Manager. If you still need assistance after reviewing these questions and answers, please contact Affymetrix Technical Support. For support contact information, see *Technical Support*, on page 6.

Q: I want to view information about other expression samples. I try to select another sample by clicking its icon, but the software doesn't change to a different sample. What should I do?

A: In GCOS Manager, click the text representing the sample or experiment name rather than the icon.

Q: How can I change the "export to" or "import from" location?

A: On the menu bar, click the icon. If you are in the **Process**, **Publish**, or **Find** tabs, this will select a target location for exported files. If you are in the **Import** tab, this will select a location to import files.

Q: How do I use the **Find** command to search the Publish database?

A: The **Find** command only works with the Process database.

Q: I know some of my samples (projects or experiments) are in the process database. However, when I use the **Find** command, the results produce an empty set. Why does this happen?

A: In the **Find** window, the **Select Object** tab, the **Advanced Filter** tab, and the **Data Filter** tab are additive. Therefore, if you select experiments with the text string "*xxxAssay*" using the **Select Object** tab and then limit the search to *Project X* using the **Advanced Filter** tab, the **Find** command searches for experiments with the text string "xxxx" that are members of Project X.

Q: I want to delete (assume ownership, export, or archive) multiple data items (experiments or samples) at one time. However I cannot select multiple data items at one time in the process database. How can I accomplish this?

A: You can SHIFT-select or Ctrl-select multiple data items at one time in the Find window. Most of the functions that are available in the **Process** tab are also available in the **Find** window.

Q: I want to archive a sample but the software will not let me choose that function. How do I archive a sample?

A: Experiments or samples cannot be archived. A list of available functions in the **Find** window is listed below:

Analysis data: archive, assume ownership, delete, and export

Experiments: assume ownership, delete, export, and rename

Samples: assume ownership, delete, and rename

Q: How do I get out of the **Find** window and back to the process database?

A: Simply close **Find** window or go to the Windows menu and select "GCOS server manager."

Q: Can I view property information about files in the publish database?

A: No, this information is not available. But you can view property information about files in the process database in the **Publish** or **Process** tab.

Q: I cannot find my image data (.dat) under the Import tab. I have confirmed that the .dat file exists on the local drive and that I have the import location mapped correctly. Why doesn't GCOS Manager let me import my image data (.dat)?

A: GCOS Manager imports .exp data and the associated image data (.dat) with the same name. Make certain that the folder with the image data (.dat) includes the .exp data with the same name. As a last resort, open the image data (.dat) in Affymetrix® GCOS. If an .exp does not exist for the image data (.dat), GCOS will automatically create one.

Q: When I try to open a Sample folder, only a few samples are displayed and I know that there should be more in the database. What happened to the samples?

A: The Sample filter remains active during successive sessions. Reset Sample Filter parameters. See *Filtering Data*, on page 65.

Library Files Troubleshooting

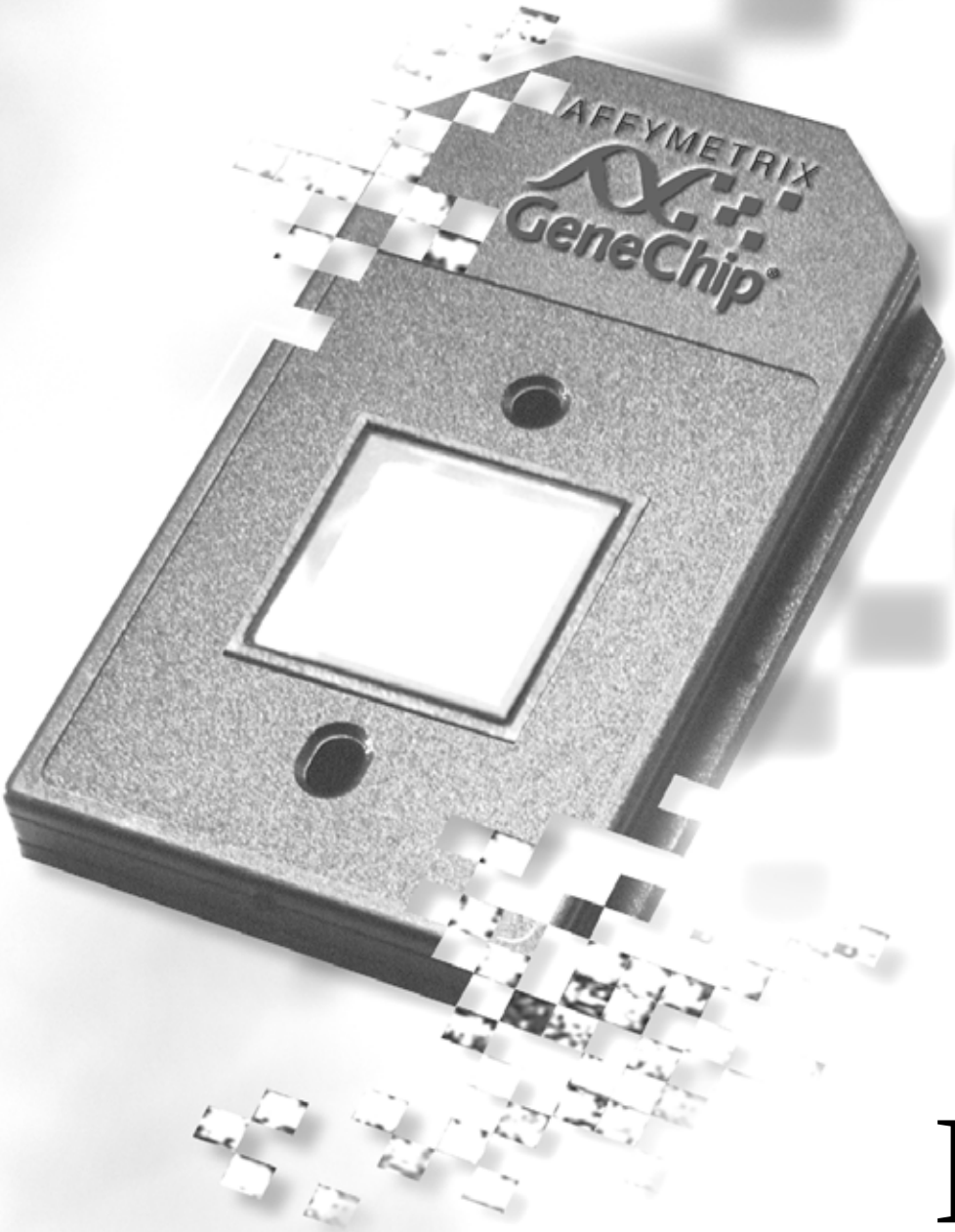
Installation

- **During the library file installation on the server, it shows that the ask is “not responding” in task manager. What is wrong?**

  The library file installation is responding. During this time, it is writing the gene descriptions and sequence information into the databases. This will take a while depending on the number of probe arrays selected. Do not stop or cancel the task.

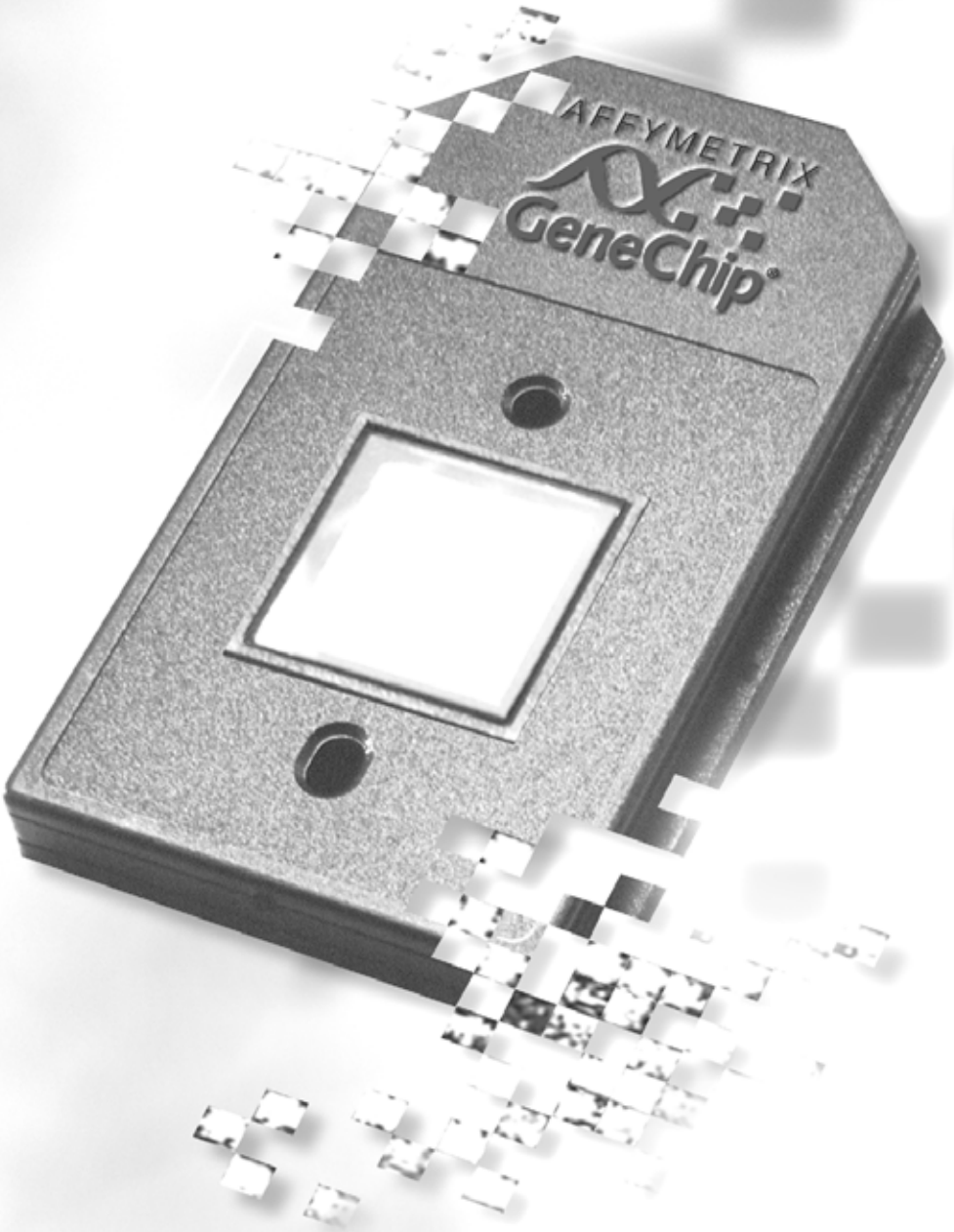
- **After an upgrade, the library files update installation is run. The installation completes very fast. Is this correct?**

  Yes. The update copies the *.cif file in the X:\GeneChipDB\Library directory and adds new parameters to the database. Descriptions and sequences are not installed.



L

Appendix L



Using the GeneChip® AutoLoader

Affymetrix has designed a new scanner addition expressly for scanning multiple GeneChip® probe arrays. This is the AutoLoader. It can scan up to 48 probe arrays automatically without operator presence.

Figure L.1
The Affymetrix® GeneChip® Scanner 3000 with AutoLoader

Setting Up the AutoLoader

The GeneChip® Scanner 3000 with an AutoLoader has the following connections on the unit **(Figure L.3)**:

- AC power connection
- Ethernet connection
- Keyboard/barcode reader connection

If you must move the scanner, disconnect all cables first.

- The instrument weight is approximately 100 pounds (45.36 Kg). Do not place it on an unstable cart, stand, or table. Failure to properly support the instrument may cause serious damage or injury and may void the warranty.

! CAUTION *Heavy object. Two people are required to lift the scanner-AutoLoader.*

Connecting the Scanner and Workstation

1. Couple the barcode reader to the keyboard using the coupling block **(Figure L.2)**. Connect the coupling block to the workstation's keyboard port **(Figure L.3)**.

Figure L.2
Coupling the barcode reader to the keyboard

2. Connect the 3-pronged electric power plug to the workstation **(Figure L.3)**.
3. Connect the dedicated Ethernet cable from the scanner to the Ethernet port that is designated on the workstation. The port is located near the bottom of the workstation **(Figure L.3)**.
4. You can connect your company's network cable to the indicated port near the center of the workstation **(Figure L.3)**.

   When you start GCOS, the software will make the proper communication connections.
5. Connect the cabling to the scanner with AutoLoader **(Figure L.4)**

! CAUTION

Do not confuse your company's network connections with the dedicated Ethernet port of the scanner-workstation. The proper scanner connection is located near the bottom of the workstation.

This 10/100 Base T Ethernet communications port is dedicated to the scanner-workstation interface. You cannot connect the scanner to your company's Ethernet communications network.

You can, however, connect the workstation's second Ethernet port to the your company's Ethernet network.

6. On the rear of the AutoLoader, there are two drain tubes: an upper and a lower drain tube. Place the upper drain tube in a beaker or other receptacle to collect the condensation from the AutoLoader **(Figure L.4)**.

✔ NOTE

The reset button is the scanner's circuit breaker. The breaker switch will be tripped whenever the scanner experiences an electrical fault condition. Press to reset. If you cannot reset this switch, contact Affymetrix technical support.

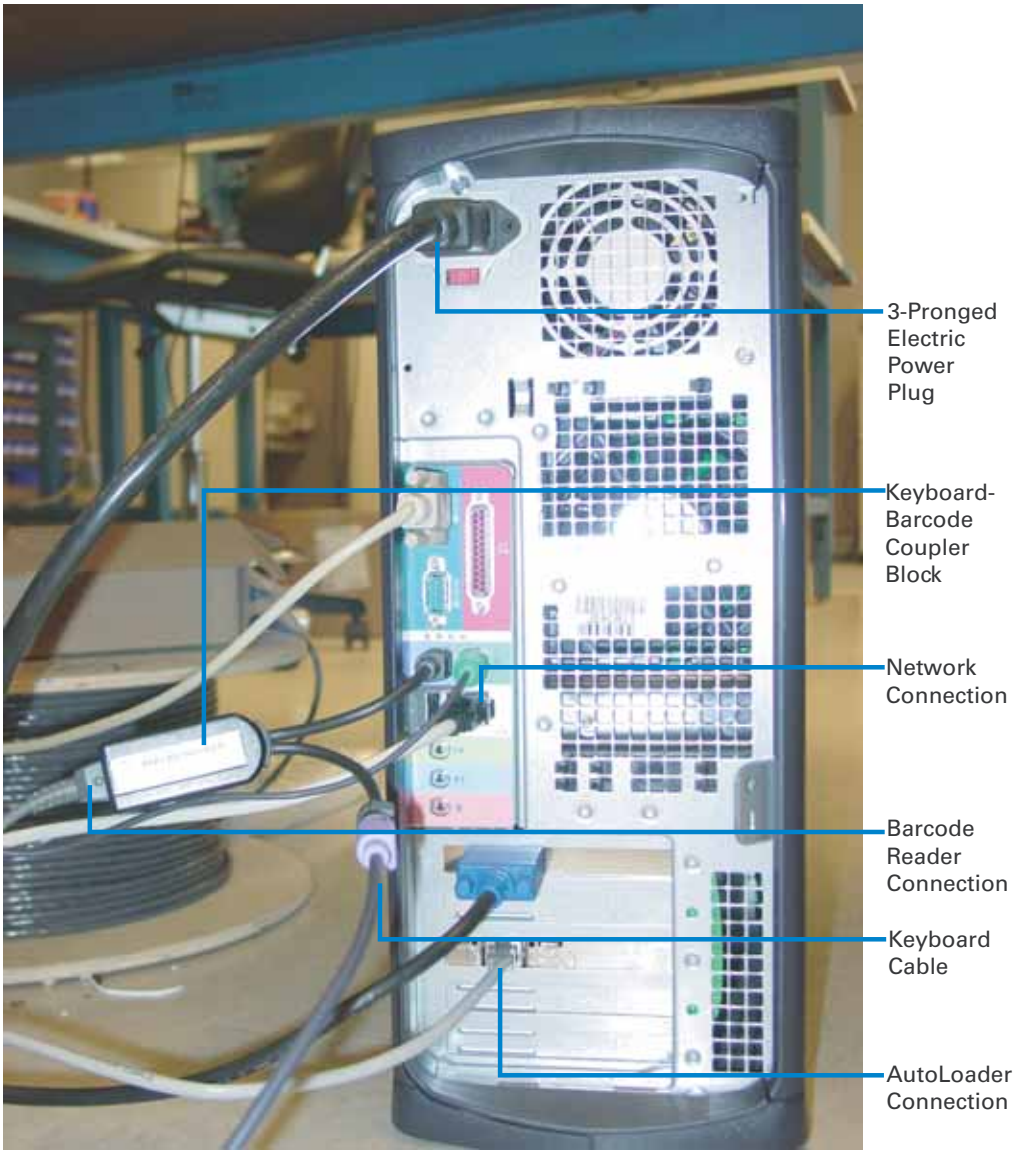


Figure L.3
Workstation rear cable connections

Figure L.4
Scanner with AutoLoader rear connections

Indicator Lights and On/Off Button

The front panel has the following button and indicators **(Figure L.5)**.

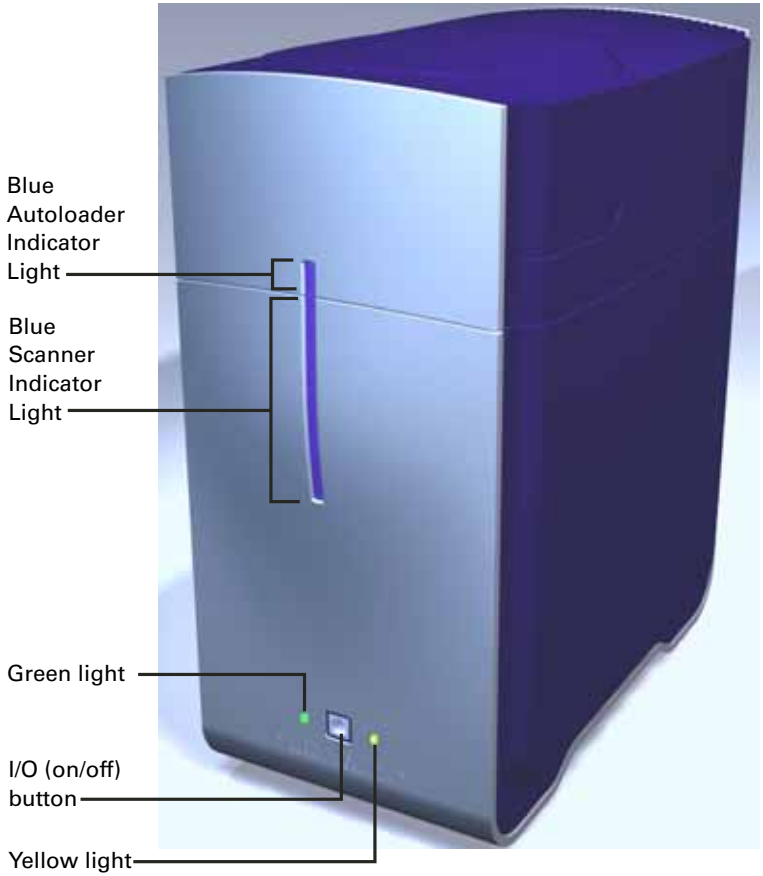


Figure L.5
The AutoLoader indicator lights

1. I/O (on/off) button in the center.
2. Blue indicator light on scanner body, running vertical at front center. This light extends to the bottom of the AutoLoader and is always on when the scanner is on.

3. Blue indicator light on AutoLoader, running vertical at front center. This light appears to be merely an extension of the scanner light. However, when on, it indicates that the AutoLoader door is closed and locked. It will turn off when the door is unlocked.
4. Green and yellow light both on indicate scanner boot up in progress.
5. Yellow light
   a. On = Idle, laser is warming up (laser not ready, green off)
   b. Off = System ready, no errors (Green on)
   c. Flashing = Error
6. Green light
   a. On = System is ready to scan (yellow off)
   b. Flashing = Scan in progress

Summary of Indicator Lights

The table below summarizes the light conditions and their meaning.

Condition	Green Light	Yellow Light	Blue Scanner Indicator Light	Blue AutoLoader Indicator Light	Meaning
Initial boot up	Off	Off	On	Off	Initial power up; embedded PC takes control
Scanner boot up	On	On	On	Off	Embedded PC takes control of scanner boot up
Laser warm up	Off	On	On	Off	Software enabled and laser is warming up
System ready	On	Off	On	Off	Scanner ready for use and AutoLoader door is unlocked waiting to receive a carousel
Error	Off	Flashing	On	Off	Fatal error, reboot scanner and software, AutoLoader door is unlocked to remove carousel if necessary
Scanning	Flashing	Off	On	On	Scanning is in progress and AutoLoader door locked
Scanning	Flashing	Off	On	Off	Scanning is in progress and AutoLoader door is unlocked

Installing and Configuring the E-mail System

In the event of an AutoLoader error condition, you can enable the software to send an e-mail containing error information using Microsoft Exchange server or SMTP.

The user sets up the e-mail system just once.

E-mail System Installation

The instrument installation software is on a separate CD. Use this CD and the following procedure to install the instrument control software.

1. Launch Windows 2000 Explorer.
2. Double-click **setup.exe** within the Instrument folder.
   ⇒ The Welcome window appears.
3. Click **Next**.
   ⇒ Several consecutive Software License Agreement windows appear.
4. Click **Yes** in each window to accept the terms of the agreement.
5. The **Choose Destination Location** window appears.
6. Click **Browse** and select the **Destination** to install the instrument driver (select the same location where you installed GCOS).
7. Click **Next**.
   ⇒ The Select Components window appears **(Figure L.6)**.

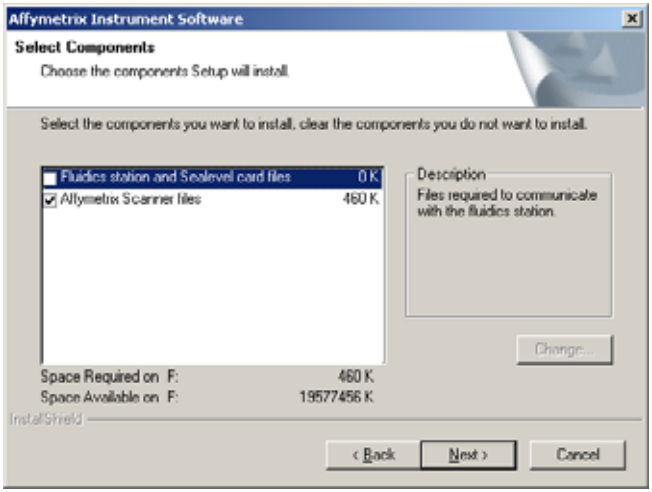


Figure L.6
The Select Components window, only the Affymetrix Scanner files box checked

8. Remove the check from the **Fluidics station and Sealevel card files** box. Select only the **Affymetrix Scanner Files** option. Highlight the **Affymetrix Scanner Files** option and click **Change...** to select the type of scanner. The following dialog box opens and displays the choice of scanners, Affymetrix or GeneArray **(Figure L.7)**.

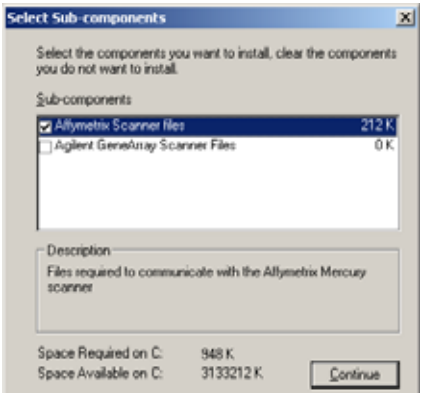


Figure L.7
The Select Sub-components window, only the Affymetrix Scanner files box checked

9. Select the **Affymetrix Scanner files** and click **Continue**.
10. Click **Next**.

    ⇒ The Email Option window appears **(Figure L.8)**.

Using Microsoft Exchange

If you are using Microsoft Outlook with Microsoft Exchange, you must configure Outlook XP to work with the Exchange server.

Each user can set up an individual distribution list, and all users share the same name for each of their own individual distribution lists. Thus there can be two cases in sending e-mail:

- There is a single e-mail contact for everybody
- There is a common distribution list name, and every user must set up a list with that name.

Configure it by the following steps.

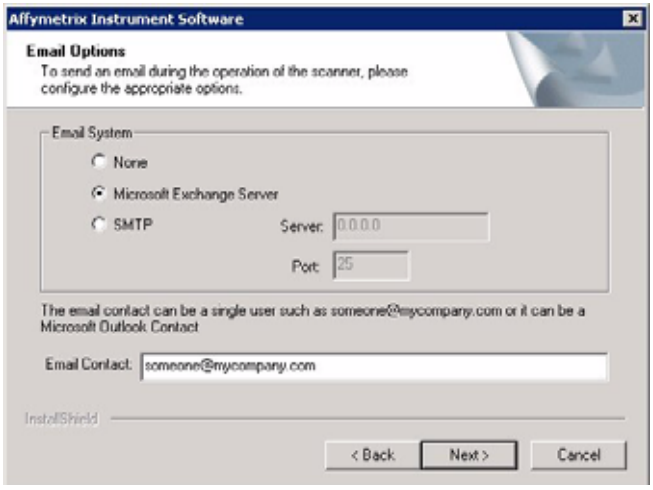


Figure L.8
The Email Option window with Microsoft Exchange Server checked

1. Select the Microsoft Exchange Server. Enter an e-mail address or a distribution list that has been set up in Outlook. To set up a distribution list see the section below.

✔ NOTE

When an e-mail is sent during a normal run, you must be logged into the workstation and have an account on the exchange server.

Setting up Outlook for a distribution list

Let us call the name of the distribution list AutoLoaderGroup.

1. Launch Microsoft Outlook.
2. On the File menu, point to New, and then click Distribution List.
3. In the Name box, type the name that is used in the Instrument Install (e.g. AutoLoaderGroup).
4. Click Select Members.
5. In "Show names from the list," choose the address book that contains the e-mail addresses you want in your distribution list.
6. In the "Type name or select from list box," type a name you want to include, or select a name from the list below, and then click Members. Do this for each person you want to add to the distribution list, and then click OK.

   If you want to add a longer description of the distribution list, click the Notes tab, and then type the text.
7. You save the distribution list in your Contacts folder by the name you give it.

   All e-mail generated during an AutoLoader run will be sent to the people in the distribution list

   Each user, who plans to run the AutoLoader through his/her user account, should create a distribution list.

Configuring SMTP

If you are using SMTP, configure it as follows.

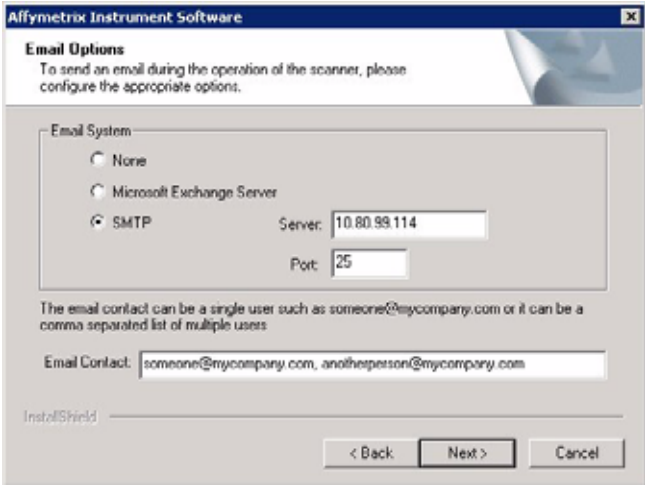


Figure L.9
The e-mail contact configuration window with SMTP configured

1. Enter the IP address of the SMTP server and the port number at which SMTP transactions occur.
2. Enter an e-mail address or comma separated list of e-mail addresses to which the e-mail must be sent.

No E-mail Options

Select this option to disable the e-mail system. Place a check in box None.

E-mail Messages

If the e-mail system is enabled, the instrument control software sends an e-mail alert for conditions that may occur during an AutoLoader run.

IMPORTANT

For a list of serious errors, see the section entitled *AutoLoader Error Messages* on page 700.

In case of a fatal error:

1. The software sends an e-mail to the specified set of e-mail addresses or to the Outlook distribution list.
2. The software provides you with the ability to send an e-mail without your intervention.
3. The software uses extended MAPI to send an e-mail.
4. Each e-mail message contains the following information:
   a. Date and Time
   b. Scanner ID
   c. All experiment information displayed in the status window. The table below lists the relevant e-mail messages **(Table L.1)**.

Table L.1
E-mail Message Conditions. These will appear on the scan status field of the status screen at the bottom of the GCOS GUI such as the Experiment Information Window. (See the section entitled *Troubleshooting* on page 699 for recommended action).

Condition	Detected By	Action/Mitigation
Carousel Home Error	Carousel Home not detected after more than one carousel rotation	Log error, stop run, notify user via GUI and e-mail
Grip Home Error	Grip Home not detected after more than full actuator travel, a mechanical error	Log error, stop run, notify user via GUI and e-mail
Feeder Fail	Cartridge not detected in scanner or AutoLoader during load or unload	Log error, stop run, notify user via GUI and e-mail
Load Request Error, Cartridge in Scanner	Cartridge already in scanner when software directs AutoLoader to load a cartridge	Log error, stop run, notify user via GUI and e-mail

Table L.1 (Continued)
E-mail Message Conditions. These will appear on the scan status field of the status screen at the bottom of the GCOS GUI such as the Experiment Information Window. (See the section entitled *Troubleshooting* on page 699 for recommended action).

Condition	Detected By	Action/Mitigation
Unload Error, Cartridge in AutoLoader	Cartridge detected already in AutoLoader when software directs AutoLoader to unload a cartridge (Autoloader cannot unload the cartridge.)	Log error, stop run, notify user via GUI and e-mail
Cooling Over Temperature	Cooling set point not attained within 1 hour of activation	Log error, disable cooling, notify user via GUI and e-mail, continue AutoLoader run
Cooling Under Temperature	Cooling temperature < 5ºC	Log error, disable cooling, notify user via GUI and e-mail, continue AutoLoader run
Door Opened	Door was opened in the middle of a scan causing current chip to be rescanned	Log error and e-mail
Power failure/restore	Power was lost/or restored to the workstation and /or AutoLoader	If a UPS is attached to the workstation, an e-mail will be sent
Network disconnect	A network disconnect between workstation and scanner was detected	Log error and e-mail user
End of Run	Scanner stops the AutoLoader run upon encountering a chip previously scanned or after scanning 48 chips	Notify user via GUI and e-mail that the run has completed

Operating the Scanner-AutoLoader

This chapter describes how to use GCOS to control the Affymetrix® GeneChip® Scanner and AutoLoader **(Figure L.10).**

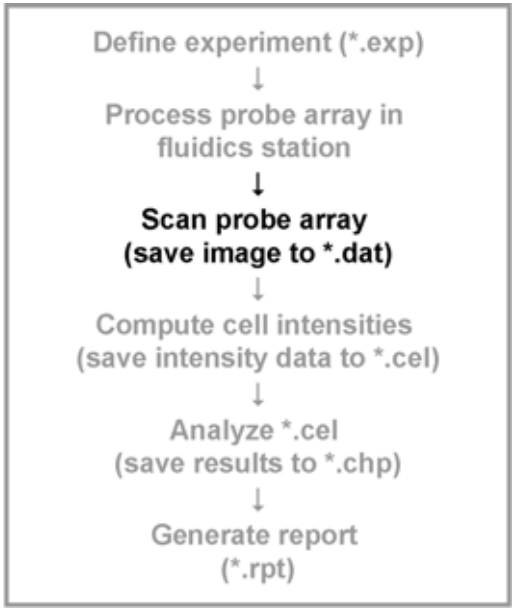
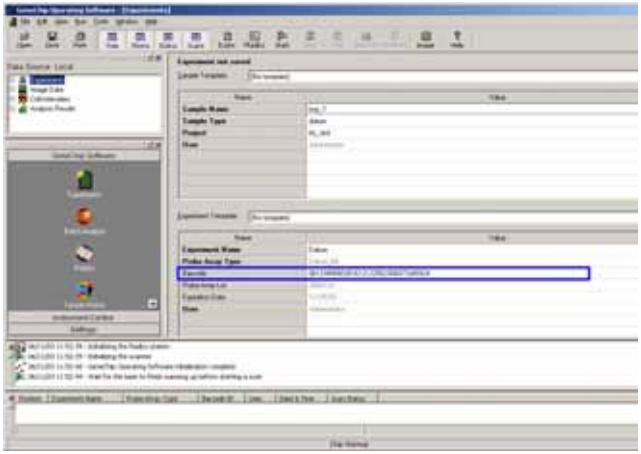


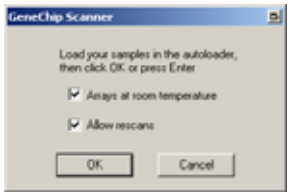
Figure L.10
Assay & analysis flow chart

Quick Reference Walkthrough

The table below illustrates the steps required to scan a set of probe arrays.

Step	Description
1. Enter and save the experiment information in the Experiment Information Window. For more information, see the section, *The Experiment Information Window*, on page 673.	
2. Hybridize the probe arrays. For more information, see the *GeneChip® Expression Analysis Technical Manual* and relevant package inserts.	

Step	Description
3. Wash the probe arrays. For more information, see the *GeneChip® Fluidics Station 450/250 User's Guide* and relevant package inserts.	
4. Apply Tough-Spots™ For more information, see the section, *Using Tough-Spots™ to Prevent Leaks*, on page 677.	
5. Load probe array cartridges into carousel. For more information, see the section, *Loading Cartridges into the Carousel*, on page 681.	

Step	Description
6. Load carousel into the AutoLoader. Set properly by turning it to set flush with housing. Close the door. For more information, see the section, *Loading the Carousel into the AutoLoader*, on page 683.	
7. Start the AutoLoader run. For more information, see the section, *Scanning a Probe Array in Automode*, on page 685.	 • If you want to scan the probe arrays without waiting for them to have warmed up, check box. • If you want to rescan currently loaded probe arrays that had been scanned in a previous run, check Allow rescans box. The .dat file from the previous run will not be overwritten.

The AutoLoader Run

This section shows you how to scan multiple GeneChip® probe arrays using the new Affymetrix® GeneChip® Scanner 3000 equipped with the AutoLoader.

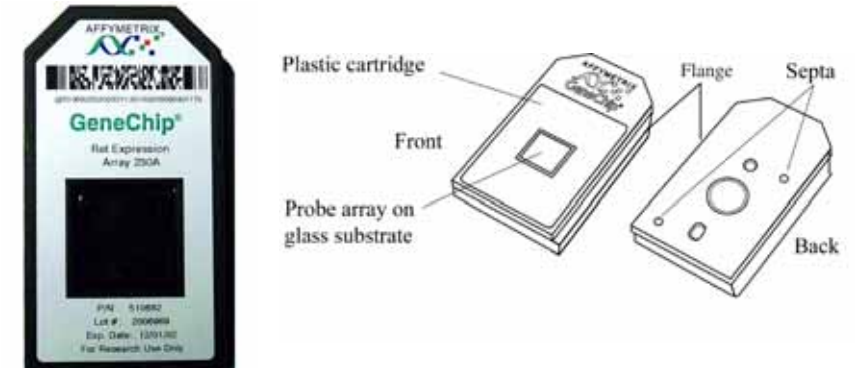
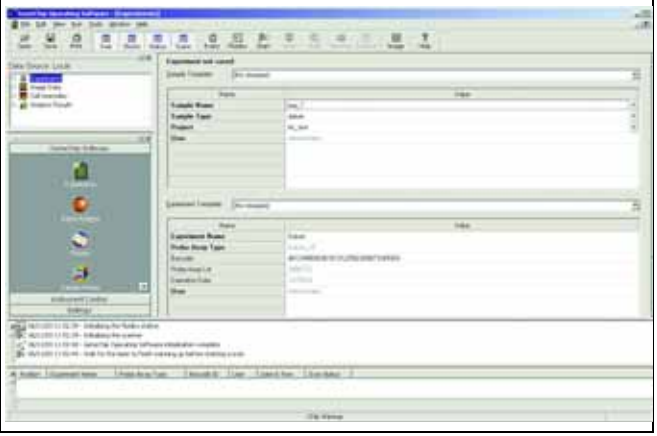


Figure L.11
Affymetrix® GeneChip® probe array cartridge: note the location of the flange. The AutoLoader will accept the cartridge in only one orientation.

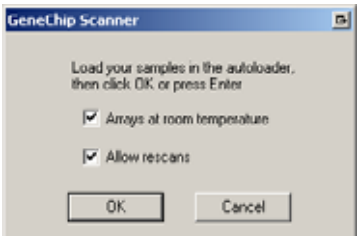
Quick Reference Guide to Using the AutoLoader

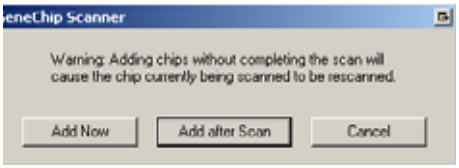
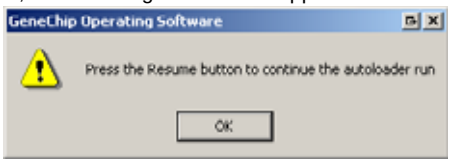
The following table can help you find specific procedures based on the type of task you are looking for.

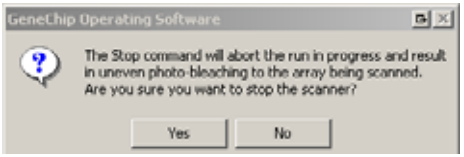
If you want to:	Then do this:
Use the AutoLoader in automode	1. Click **Tools** →**Defaults**. 2. Clear the check box **Enable Manual Mode**. 3. Click **OK** **(Figure L.21)**.
Use the AutoLoader in manual mode	1. Click **Tools** →**Defaults**. 2. Check **Enable Manual Mode**. 3. Click **OK** **(Figure L.24).**

If you want to:	Then do this:
Turn on the laser immediately when you launch GCOS	1. Click **Tools** →**Defaults**. 2. Check **Turn on Laser at startup**. 3. Click **OK** **(Figure L.30).**
Add an experiment	1. Open the GCOS software, and click Experiments. to open the Experiment Information window. 2. Enter and save the experiment information in the Experiment Information Window. 

If you want to:	Then do this:
Add a barcode • Note: A new experiment must be opened for each barcode.	1. Open the GCOS software to the Experiment Information window. 2. Place cursor in the barcode field. Name / Value **Experiment Name** 763 **Probe Array Type** HG-U133A Barcode Probe Array Lot 2006969 Expiration Date 12/01/02 **User** emcken 3. Hold a GeneChip probe array cartridge in front of the barcode reader and squeeze the trigger for up to four seconds until you hear a beep. The reader reads and sends the barcode to the GCOS Experiment Information window, Barcode field. The software automatically fills other fields. Name / Value **Experiment Name** 763 **Probe Array Type** HG-U133A Barcode [illegible] Probe Array Lot 2006969 Expiration Date 12/01/02 **User** emcken 4. After the software adds the barcode, save the experiment. 5. Repeat steps 1 to 4 until you have read all of the probe array cartridges.

If you want to:	Then do this:
Start a scanning run	1. Load the carousel and place the carousel into the AutoLoader. 2. Click the **Start** button . • If the arrays are at room temperature, or you want to scan the probe arrays without waiting for them to warm up, check the appropriate box. • If you want to allow the probe arrays to be rescanned, check the appropriate box.  (The rescan function is handy if you want to rescan a currently loaded probe array that had been scanned in a previous run.) 3. Click **OK**. The AutoLoader homes and performs an inventory of the probe arrays and the scanning run begins.

If you want to:	Then do this:
Add a probe array during a scan	1. Click the Add button or select **Run →Add Chips**.  • If you want to unlock the door and immediately begin adding cartridges, click the **Add Now** button. The blue indicator light on the front of the AutoLoader will go out signifying that the door is unlocked and you can load more cartridges. **The cartridge being scanned, if any, will be rescanned, and the previously created .dat file WILL BE OVERWRITTEN.** • If you want to wait for the scan to complete before adding a probe array, click the **Add after Scan** button. The current scan will continue until completion, then the door will unlock and you can load more cartridges. The Autoloader blue indicator light will go out to signify that the door is unlocked. • In both cases, after you have added your cartridges and closed the door, the following window will appear. Click **OK**.  2. Click the Resume button or **Run →Resume**. The door then locks and the blue indicator light turns on.
Use GCOS without a scanner	1. Click **Tools →Defaults**. 2. Clear the check box **Scanner Installed**. 3. Click **OK** **(Figure L.28).**
Disable AutoLoader and use the scanner only	1. Click **Tools →Defaults**. 2. Check **Disable AutoLoader**. 3. Click **OK**. **(Figure L.29).**

If you want to:	Then do this:
Stop a scan	1. Click the Stop button . 2. When the following window appears, click **OK**.  **Caution: If you stop the scanner while a probe array is in the process of scanning, you will lose all scan information from that probe array. If you rescan the array, it may be affected due to uneven photo-bleaching. This could potentially make the data from the array difficult to compare to other array data.**

Terminating a scan run

The AutoLoader run will terminate under certain normal circumstances. The following table **Table L.2** outlines under what conditions a scan run will or will not terminate.

Table L.2
Summary of scan run termination conditions

The scan run will terminate if:	The scan run will not terminate if:
You press the Stop button. Caution: If you stop the scanner while a probe array is in the process, you will lose all scan information from that probe array. If you rescan the array, it may be affected due to uneven photo-bleaching. This could potentially make the data from the array difficult to compare to other array data.	You check Allow Rescans box. When the AutoLoader encounters a probe array that was previously scanned in an earlier run, it will rescan that probe array and will create additional .dat files (.dat1, .dat2, etc.).
The AutoLoader detects a probe array with the same barcode in the current run, i.e., in the currently loaded carousel. The probe array will not be rescanned.	You clear the Allow Rescans box. When the AutoLoader encounters a probe array that was previously scanned in an earlier run, it will log the probe array but will not rescan it. It will continue the run.

Table L.2 (Continued)
Summary of scan run termination conditions

The scan run will terminate if:	The scan run will not terminate if:
The AutoLoader detects 48 scanned cartridges in the same run.	You click the Add Now button. The AutoLoader door will unlock to accept new probe array cartridges. The scan in progress will complete. When you close the door and continue, the AutoLoader will home, take inventory and move to the same probe array that was in the process of being scanned when the door was opened. It will discard the earlier .dat file and rescan that probe array. Note: this has nothing to do with the Allow Rescans check box.
	You click the Add After Scan button. The AutoLoader door will wait until the scan in progress is complete then unlock the door. When you close the door and continue, the AutoLoader will home, take inventory and move to the next probe array from that which was in the process of being scanned when the door was opened.

The Experiment Information Window

The Experiment Information window (EIW) provides you with the means for generating and saving a database of your experimental data **(Figure L.15)**.

✔ NOTE *Before you can advance to the next experiment, you must first save the current experiment.*

Certain information has to be entered before the experiment can be saved. **(Table L.3)** lists the available fields and their definitions and whether or not they are required in order to save the experimental data.

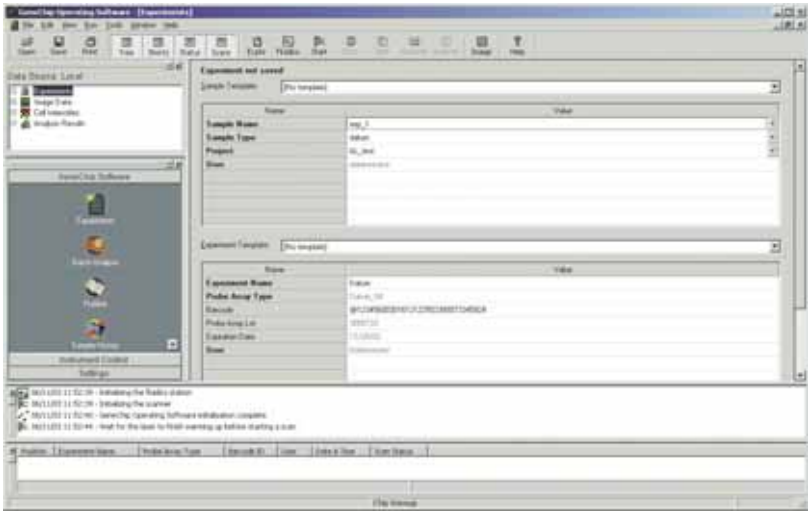
For more information, see the section, *Registering a Sample & Defining an Experiment*, on page 74.

Table L.3
Definitions of the experiment data fields in the Experiment Information Window

Field	Definition	Required for save
Sample Template	Dropdown box for templates associated with samples	No
Sample Name	The name of the sample.	***Yes***
Sample Type	The type of sample. This is an attribute of the sample that describes its type. Example: blood, cell line, tissue, etc.	***Yes***
Sample Project	The name of the project associated with the sample.	***Yes***
Sample owner	Read-only field showing the owner of the sample in the database.	***Yes***
Experiment Template	Dropdown box for experiment templates	No
Experiment Name	The name of the experiment.	***Yes***
Probe Array Type	This is the probe array type associated with the experiment, and automatically filled in when you enter the barcode.	***Yes***
Barcode	User entered barcode	***Yes (No if AutoLoader is in manual mode)***
Expiration Date	Array Expiration date embedded in Affymetrix barcode, and automatically filled in when you enter the barcode.	No
Probe array Lot number	Array lot number embedded in Affymetrix barcode, and automatically filled in when you enter the barcode.	No
Experiment owner	Read-only field showing experiment owner	***Yes***
User-set	Drop down list of user-sets in the database used for storing analysis parameters	No
Publish Database	Drop down list of names of the publish databases. Used when selecting automation	No
Publish Intensities	Checkbox to be selected if publishing intensities to the database after completing the analysis	No

Status Window

GCOS software provides you with the status of all scans, the status of the AutoLoader door and status of the current scan. This window is located at the bottom of user interface **(Figure L.12)**. Below **(Table L.4)** describes the status fields.



Status window

Figure L.12
The status window

Table L.4
The GCOS Status Fields and Descriptions

Status Field	Description
Position	Position occupied by a given cartridge in the AutoLoader carousel
Experiment Name	The experiment name associated with the scan associated with a given cartridge position
Probe Array Type	The probe array type for the scan associated with a given cartridge position
Barcode ID	The unique identifier in the barcode for the scan associated with a given cartridge position

Table L.4 (Continued)
The GCOS Status Fields and Descriptions

Status Field	Description
User	Name of the user (experiment owner) for the scan associated with a given cartridge position
Time & Date	The date and time when scan started and completed
Scan Status	The status of the scan. (Autofocus, scanning). This field displays all scanner status strings associated with the scan and retrieved from the scanner. The message strings that may appear in this field are listed below. Note: Not all of these messages will appear in each AutoLoader run. **Status Field Strings** Autofocus Scan Status - % of lines scanned Scan Complete status Grid alignment errors Autofocus Errors The experiment XXX has already been scanned Chip load failures Invalid barcode errors Experiment does not exist errors AutoLoader door open errors
AutoLoader Door (viewed in status bar)	The status of the AutoLoader door
Number of Cartridges (viewed in status bar)	Number of cartridges in the AutoLoader carousel as determined by inventory
History (viewed as a log)	A running history of the last 99 arrays scanned and the information on the current array (if any) being scanned

Turning on the Scanner-AutoLoader

Press the on/off (I/O) switch on the front panel. The scanner's onboard computer will boot up. The bootup process takes a few of minutes. During this time both the yellow and green light will be on. The scanner enters the laser warm-up state. During this warm-up time, the green light will turn off and the yellow light will remain on. You must wait 10 minutes for the laser to stabilize.

Using Tough-Spots™ to Prevent Leaks

Tough-Spots™ are chemically inert polyvinyl labels that adhere to all plastics. Affymetrix recommends using 3/8-inch circle diameter Tough-Spots to prevent leakage from the cartridge septa.

Before loading the probe array cartridge, follow this procedure to prevent the leaking of fluids from the cartridge during scanning.

Even if you have already applied Tough-Spots to the cartridge prior to hybridization or after washing, you must remove the old Tough-Spots and apply new ones before you load them into the AutoLoader.

> Affymetrix recommends the use of Tough-Spots™ obtained from Affymetrix P/N 64-0158
>
> or from
>
> USA Scientific, Inc. P.O. Box 3565 Ocala, FL 34478
> (800)LAB-TIPS P/N 9185-0000

IMPORTANT *To reduce the risk of leakage, do not use excessively large pipette tips to pierce the septa.*

1. On the back of the probe array cartridge, clean excess fluid from around septa **(Figure L.13)**.

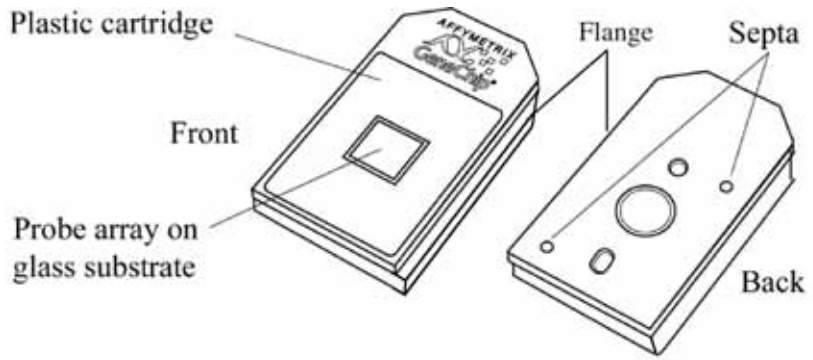


Figure L.13
The GeneChip® probe array cartridge

2. Carefully apply one Tough-Spot over each of the two septa. Press to ensure that the spots remain flat. If a Tough-Spot does not apply smoothly; that is, if you observe bumps, bubbles, tears or curled edges, do not attempt to smooth them out. Remove the spot and apply a new one **(Figure L.14)**.

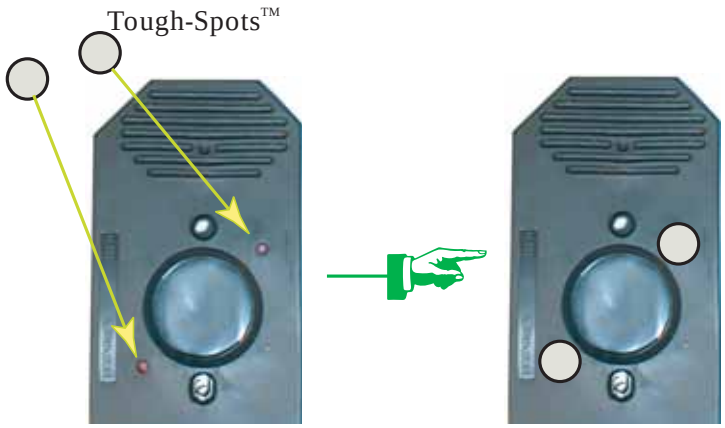


Figure L.14
Applying Tough-Spots™ to cartridge septa

Using the Barcode Reader

If you have not already done this during the initial experiment setup, you must now add a new barcode for each experiment.

1. Open the GCOS software to the Experiment Information window and place the cursor in the barcode reference field of the GCOS Experiment Information window **(Figure L.15)**.

IMPORTANT

You should open a new experiment and place the cursor into the barcode field before triggering the reader. Otherwise, the barcode will be misinterpreted as some other kind of input. A new experiment has to be opened for each barcode, and certain other information has to be entered before the experiment can be saved.

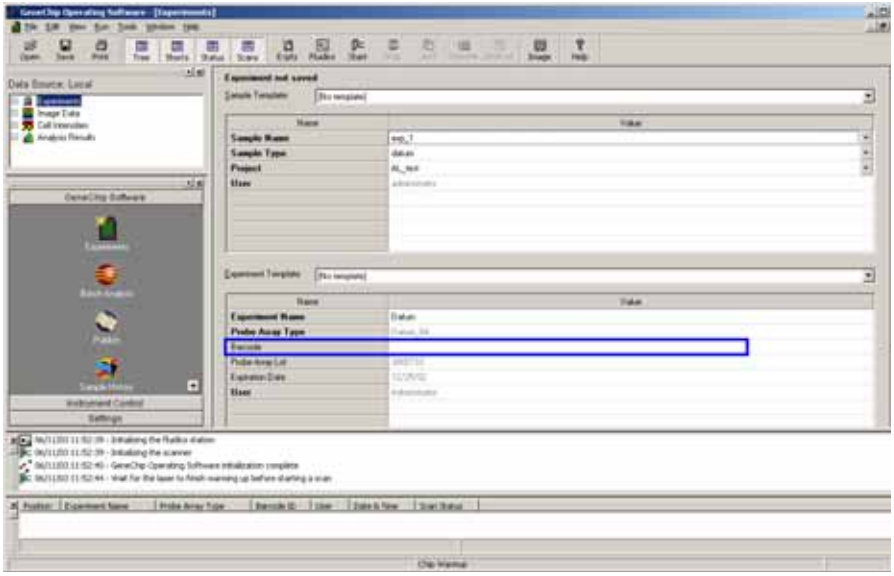


Figure L.15
Experiment Information Window with cursor in the barcode field

2. Hold a GeneChip probe array cartridge in front of the barcode reader and squeeze the trigger for up to four seconds until you hear a beep **(Figure L.16)**.

Figure L.16
Reading the cartridge barcode

3. The reader reads and sends the barcode to the GCOS Experiment Information window, Barcode field **(Figure L.17)**.

   The software also automatically fills in the “Probe Array Type,” “Probe Array Lot,” and “Expiration Date” fields. These are read-only fields.

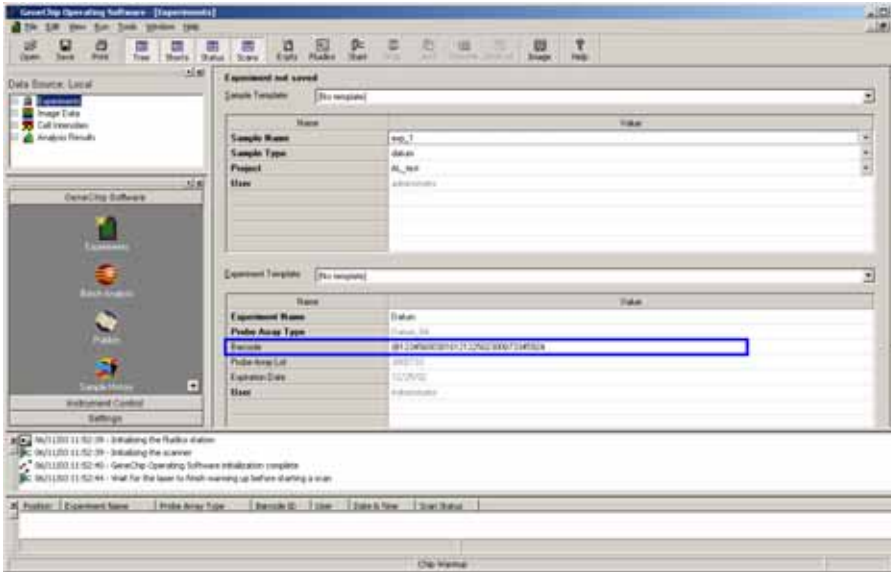


Figure L.17

4. After the software adds the barcode, save the experiment.
5. Repeat steps 1 to 4 until all of the probe array cartridges have been read.

Loading Cartridges into the Carousel

1. Load your cartridges into the carousel (up to 48). Note that only one orientation is possible **(Figure L.18)**.

   Cartridges should be loaded into the carousel starting at position #1. Additional cartridges need not be contiguous. A run will stop after 48 cartridges OR when the same barcode is read within the same run.

Figure L.18
Loading the cartridge into the chip carousel, note that each slot is numbered., 1 through 48, and each cartridge can fit in only one orientation

Loading the Carousel into the AutoLoader

1. Load the carousel into the AutoLoader by inserting the carousel into the AutoLoader and turning the carousel until the alignment pin seats into the alignment hole **(Figure L.19)**.

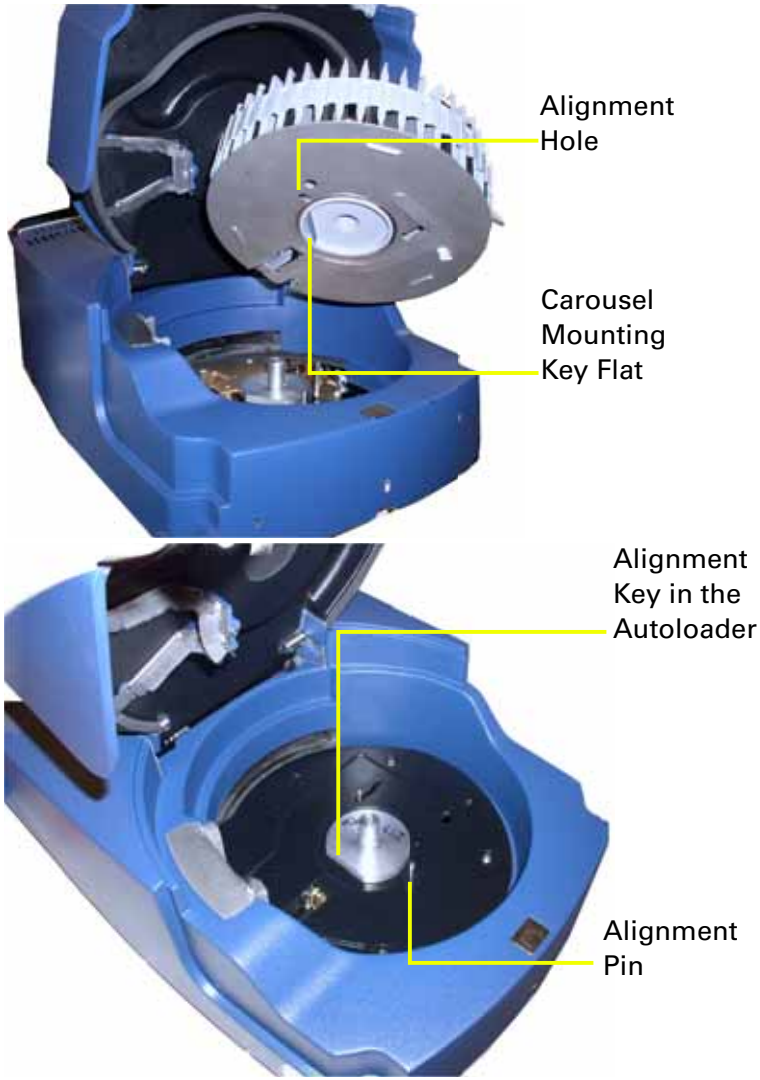


Figure L.19
Loading the cartridge carousel into the AutoLoader

2. Turn the carousel clockwise until the carousel mounting key flat seats gently into the AutoLoader alignment key. You may have to turn the carousel several times before it will seat into the alignment pin and alignment key. When seated properly, the carousel will be flush with the AutoLoader housing. Close the AutoLoader door **(Figure L.20)**.

Figure L.20
Inserting and turning the carousel; the carousel should be seated and flush with housing.

✔ NOTE *The seating of the key flat is confirmed by a gentle falling of the carousel into the key.*

Scanning a Probe Array in Automode

If all probe arrays have valid, associated barcodes, you can run the AutoLoader in automode.

✔ NOTE

If there exists an identical barcode within the database from an earlier AutoLoader run, and you want to rescan the current probe array with that same barcode, check the Allow Rescans box **(Figure L.22)**. *This will create additional .dat files. The original .dat file WILL NOT BE OVERWRITTEN.*

However, if the AutoLoader encounters the same barcode within the same run, the run will terminate.

1. Set the default settings.
   a. Click **Tools →Defaults** **(Figure L.21)**.
   b. ***Clear*** the check box of **Enable Manual Mode**.
   c. Click **OK**.

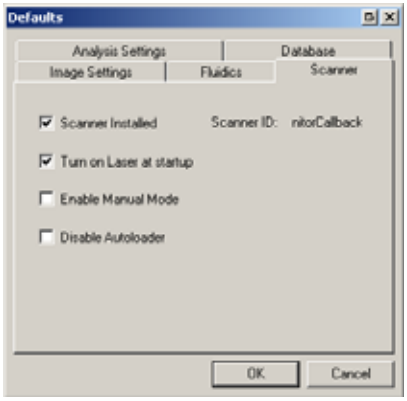


Figure L.21
Defaults window showing "Enable Manual Mode" with the check box cleared

2. Click the **Start** button in the Instrument Control shortcut bar or the main toolbar. Alternatively, select **Run →Start Scanner** from the menu bar.

   ⇒ The Start window appears **(Figure L.22)**.

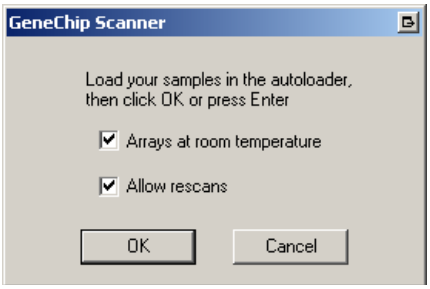


Figure L.22
The Start Scanner window in automode

If you have not already loaded your probe array cartridges, do so now.

If you want to skip the ten minute warm-up period before scanning the first probe array, keep the default check in the **Arrays at room temperature** box.

3. Click **OK** in the GeneChip Scanner dialog box to start the run.
   - The AutoLoader blue indicator light will light up signifying that the AutoLoader door is now locked.
   - The carousel automatically homes itself and performs inventory to determine the number and position of cartridges present.
   - The scanning run begins. During the scan, the green light will flash, and the yellow light will be off.
   - The AutoLoader completes the autofocus operation before scanning each of the probe arrays. This takes approximately two to three minutes. The scanner cannot be stopped during this period.
   - The run will stop automatically when the last cartridge is scanned.
   - At the completion of each scan, the GCOS software will attempt grid alignment. If it is successful, the scan data will be automatically advanced to the Grid Alignment processing state.
   - If the View Scan In Progress feature is enabled (select **View →Scan in Progress** from the menu bar), the Image window automatically opens in the main display area when a scan starts. It displays the fluorescence intensity of the probe array at approximately 200 lines at a time as the scan progresses.

- To enable (or disable) this option, select **View** from the menu bar and place (or remove) a check mark next to **Scan in Progress**.

After the scan is completed, GCOS:

a. saves the image data to an image data file (*.dat) (displayed in the main display area)

b. aligns a grid on the *.dat to identify the probe cells

c. automatically computes probe cell intensities and saves the data to the cell intensity file (*.cel)

d. ejects the probe array.

- The AutoLoader will **skip** probe array cartridges if the AutoLoader encounters:

a. cartridges with unreadable or invalid barcodes or without barcodes

b. cartridges with barcodes that are not associated with an experiment

c. cartridges that have been previously scanned if the **Allow Rescans** checkbox is cleared **(Figure L.22)**.

✔ NOTE

AUTOROTATION

The AutoLoader is equipped with a heater to warm up the cartridges prior to scanning in order to reduce condensation and fogging of the probe array cartridge window.

The autorotation routine is used for temperature stability but only after the AutoLoader run is complete or during a power failure as described below.

Autorotation occurs during a power failure only if the uninterrupted power supply (UPS) is included as an accessory. The UPS provides power to the scanner/AutoLoader during a power failure. If the power fails during the scan of an array, that scan is completed and then the system turns off the heater and enters the autorotation mode to conserve power and cool the chips in the carousel. The system will also attempt to send an e-mail to notify the user of the power failure.

During an AutoLoader run, the carousel is rotating as the chips are processed to introduce the next chip to the scanner, so autorotation is not needed. After the AutoLoader run is complete the heater is turned off and the carousel is rotated to get even cooling of the chips.

Adding Cartridges during a run

The software provides you with a button that will allow you to unlock the AutoLoader door and add additional probe array cartridges while in the middle of an AutoLoader run. You have two choices: to add cartridges immediately even while a scan is in progress (in which case the .dat file is discarded and replaced with a newly collected one after the AutoLoader run is resumed), or, as a second choice, to add cartridges after a scan has completed (in which case the .dat file is saved).

✔ NOTE *The Add Chips button is enabled only after the AutoLoader has started a run in automatic mode.*

Click the **Add Chips** button , or select **Run →Add Chips** from the menu bar.

⇒ The software displays a dialog box with three buttons (1) Add Now (2) Add after scan is complete (3) Cancel **(Figure L.23)**.

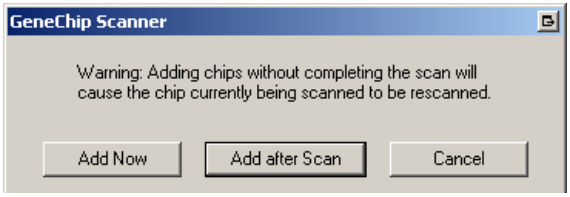


Figure L.23
The Add Chips selection buttons

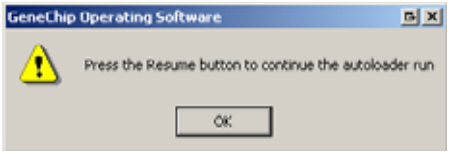
1. If you click the **Add Now** button:

   a. The blue AutoLoader indicator light will turn off signifying that the AutoLoader door is now unlocked. Open the door and add probe array cartridges.

      If a scan is in progress, the software will continue to record the scan. However, to avoid the possibility of generating a corrupt .dat file, it will discard that scan that was in progress when the door was opened.

✔ NOTE *The Resume button* *is available only for the Add Chips procedure.*

b. After you have added your cartridges, close the door and the following window appears.



c. Click **OK** and then click the Resume button or select **Run** → **Resume**.

The blue AutoLoader indicator light turns on signifying that the door is locked. The software homes the carousel and inventories the number of present probe arrays. **The carousel then moves to that last previously scanned probe array (when the door was opened) and continues the scanning run by rescanning that probe array cartridge**.

The cartridge is rescanned and a new .dat file overwrites the previous .dat file.

IMPORTANT

Do not load a cartridge into the same carousel position that was occupied by the cartridge that had just been scanned, i.e., do not replace cartridges.

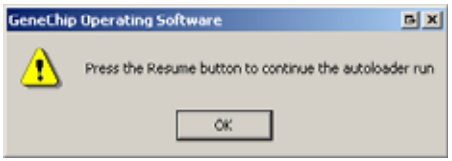
2. If you click the **Add after scan is complete** button,

a. The AutoLoader will wait until the current probe array cartridge has undergone the autofocus and scan procedures before unlocking the door to allow you to add probe array cartridges.

b. The blue AutoLoader indicator light will turn off signifying that the AutoLoader door is now unlocked.

c. Open the door and add probe array cartridges.

d. After you have added your cartridges, close the door and the following window appears.



The GCOS status bar will also display a "waiting to start" status.

e. Click **OK** and then click the Resume button or select **Run** → **Resume**.

The blue AutoLoader indicator light turns on signifying that the door is locked. The software homes the carousel and takes inventory of the probe array cartridges present. **The carousel then proceeds to the next cartridge position following the previously scanned probe array.** The AutoLoader continues the run from that cartridge position.

3. If you click the **Cancel** button, the AutoLoader will continue the AutoLoader run.

Scanning a Probe Array in Manual Mode

In the AutoLoader Automode, each probe array cartridge requires a valid barcode in order to be scanned. The manual mode feature allows you to scan one probe array at a time without the requirement of a barcode. This is useful if you must scan probe arrays that have invalid or absent barcodes.

1. Set the default settings.
   a. Click **Tools** →**Defaults** **(Figure L.24)**.
   b. Check **Enable Manual Mode**.
   c. Click **OK**.

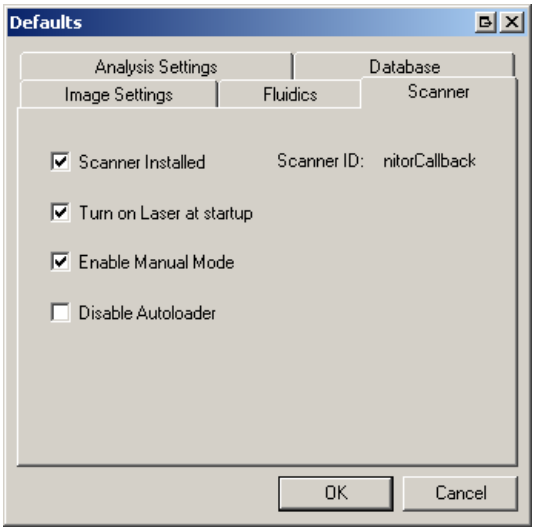


Figure L.24
Enable Manual Mode checked

2. Click the **Start** button in the Instrument Control shortcut bar or the main toolbar. Alternatively, select **Run →Start AutoLoader** from the menu bar **(Figure L.25)**.

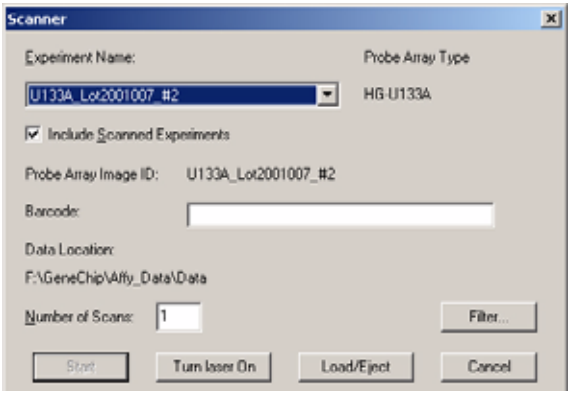


Figure L.25
The Scanner control window

⇒ The scanner Start window appears. At this point, you can choose from a number of options:

1. You can click **Start** without selecting an experiment. If your cartridge has a valid barcode, the software will get the barcode from the cartridge in the AutoLoader and select the correct experiment.
2. If your cartridge has a valid barcode, you can scan the barcode on the probe array cartridge in to the barcode field. The software will retrieve the experiment associated with the array.
3. If your cartridge does not have a valid barcode, you can manually select an experiment as described below.
   a. The **Experiment Name** drop-down list displays the experiments (*.exp) in the current directory that have not been scanned (no *.dat file containing scan data exists for the experiments).
   b. To include scanned experiments in the **Experiment Name** drop-down list, choose the **Include Scanned Experiments** option.

✔ NOTE

*If filters have been applied in GCOS Server mode, the drop-down list may not include all experiments. Click **Filter** to open the **Filters** dialog box and view or specify filters.*

c. Select the experiment name of the probe array to be scanned from the **Experiment Name** drop-down list.

- The **Probe Array Type** field automatically displays the probe array type that was entered during experiment setup. GCOS sets the **Number of Scans** per probe array type (one for the GeneChip® Scanner 3000).
- You may change the number of scans in the **Scanner** dialog box **(Figure L.25)**. Multiple scans are statistically averaged to create a single image data file (*.dat).

✔ NOTE *Increasing the number of scans increases the scan time as well as the amount of fluorophore bleaching and may result in lower fluorescence intensities.*

d. Click **Start**.

⇒ The Scanner dialog box appears **(Figure L.26)**.

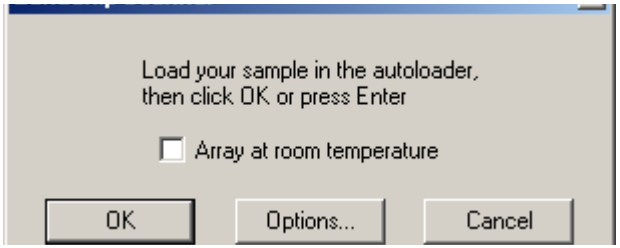


Figure L.26
The start scanner window in manual mode

- You can check **Array at room temperature** if you want to skip the ten-minute warm-up period before scanning the probe array. The default has the box already checked.

✔ NOTE *You can eject or load a cartridge by clicking on the **Load/Eject** button at any time except during the time the scanner is engaged in the autofocus operation or the scanning run.*

4. Load the probe array cartridge into slot number 1.

IMPORTANT *The cartridge slot at position number 1 is the only slot available in Manual Mode.*

5. Click **OK** in the Start Scanner box **(Figure L.26)** to start the autofocus routine. This takes approximately two to three minutes. The scanner cannot be stopped during this period.

   During the scan, the green light will flash, and the yellow light will be off.

   If the View Scan In Progress feature is enabled (select **View →Scan in Progress** from the menu bar), the Image window automatically opens in the main display area when a scan starts. It displays the fluorescence intensity of the probe array at approximately 200 lines at a time as the scan progresses.

   To enable (or disable) this option, select **View** from the menu bar and place (or remove) a check in the box next to **Scan in Progress**.

   After the scan is completed, GCOS:

   a. saves the image data to an image data file (*.dat) (displayed in the main display area)

   b. aligns a grid on the *.dat to identify the probe cells

   c. automatically computes probe cell intensities and saves the data to the cell intensity file (*.cel)

   d. ejects the probe array.

Ejecting a probe array cartridge

The probe array cartridge will automatically eject after a run; however, if you must eject a cartridge, click the Load/Eject button **(Figure L.25)**. This command is only available in manual mode.

This completes the manual mode section.

Stopping an AutoLoader Run

The Stop button is only available after you have clicked the Start button. Click this button if you want to abort a scan or run in progress.

! CAUTION

If you stop the scanner while a probe array is in the process, you will lose all scan information from that probe array. If you rescan the array, it may be affected due to uneven photo-bleaching. This could potentially make the data from the array difficult to compare to other array data.

1. Click the **STOP** toolbar button or select **Run →Stop Scanner** from the menu bar.
2. At the prompt, click **Yes** to stop the scanner or **No** to elect not to stop the AutoLoader run **(Figure L.27)**.

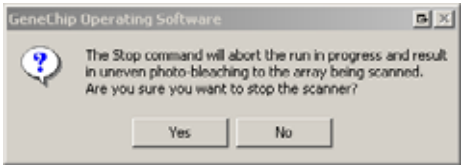


Figure L.27
Stop AutoLoader prompt

3. A window will display the message "The scanner will not stop until autofocus has finished." Click **OK**. After you stop a scan, the scanner will automatically eject the cartridge.

Shutting Down the Scanner

1. Close the GCOS software. This is the best way to shut off the laser.
2. Press the I/O button on the front panel to turn off the instrument.

Using GCOS without a Scanner.

In the event that you would like to use GCOS and do not have or do not want to attach a scanner, you should clear the check box of the Scanner Installed option in the Defaults window. This will prevent the software from continually attempting to make contact with the scanner.

1. Click **Tools →Defaults**.
2. Clear the check box of **Scanner Installed** **(Figure L.28)**.
3. Click **OK**.

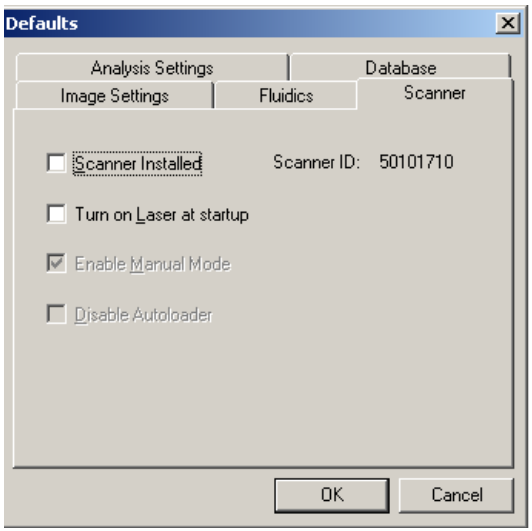


Figure L.28
Scanner Installed with the check box cleared

Using GCOS with the AutoLoader Disabled.

If you have a working scanner but the AutoLoader is not operating, you can still use the scanner, but you must check the Disable AutoLoader option in the Defaults window. This will disable the AutoLoader and enable you to use the scanner alone as you would in manual mode.

1. Click **Tools →Defaults**.
2. Check **Disable AutoLoader**.
3. Click **OK**. **(Figure L.29).**

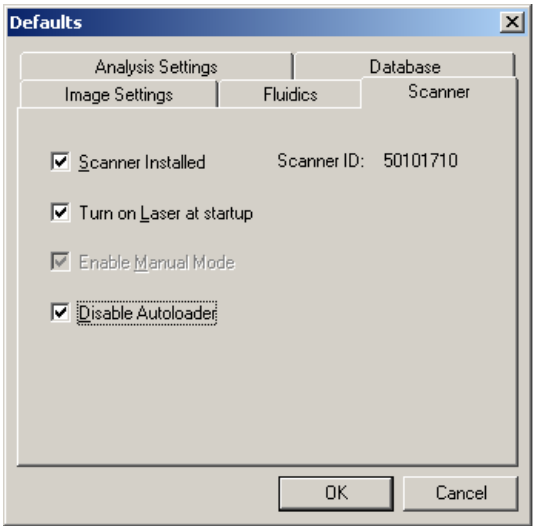
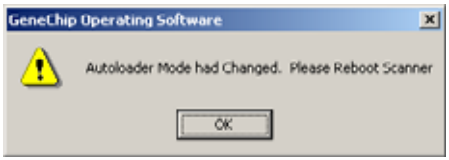


Figure L.29
Disable AutoLoader checked

⇒ A window will appear asking that you reboot the scanner.



4. Press the scanner front panel I/O button once to turn off the scanner **(Figure L.5)**. Wait a few moments, the press the I/O button to turn on the scanner.
5. Open the AutoLoader door.
6. Manually load a probe array cartridge into the slot.
7. Close the AutoLoader door.
8. Scan the probe array in the same manner as the AutoLoader in manual mode. See the section entitled *Scanning a Probe Array in Manual Mode* on page 690.
9. After scanning the probe array, you must manually remove the probe array cartridge from the AutoLoader. See Steps 7 though 13 in the the

section entitled *Manually Removing a Lodged Probe Array Cartridge* on page 701.

Turning on the Laser at GCOS Launch

You have two options as to how to control the actuation of the laser. You can have the GCOS software turn on the laser at the launch of the GCOS software. This allows you to begin the ten-minute warmup period immediately. Or you can wait until you are about to run a series of probe array cartridges to turn on the laser. You still wait ten minutes for the laser to warm up.

1. Click **Tools →Defaults** **(Figure L.30)**.
   a. If you want to turn on the laser immediately upon launching the GCOS software, check **Turn on Laser at startup**.
   b. If you want to wait until you are ready to run a scanning procedure, before turning on the laser, clear the check box for **Turn on Laser at startup**.
2. Click **OK**.

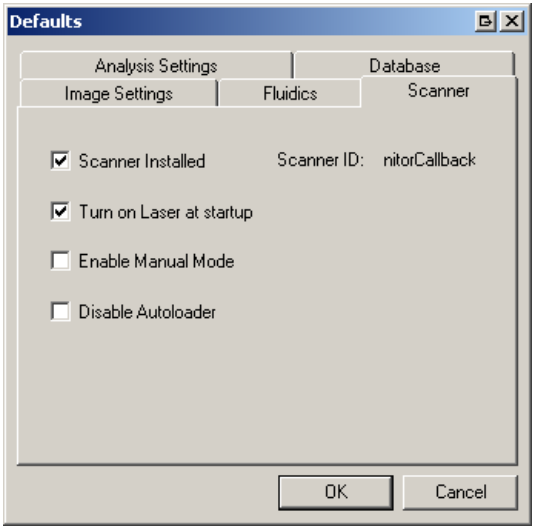


Figure L.30
Turn on Laser at startup checked

Cleaning and Maintenance

The AutoLoader requires little in the way of customer maintenance. The instrument must be kept clean and free of dust. Dust buildup can degrade performance. Wipe the exterior surfaces clean using a mild dish detergent solution in water. Do not use ammonia based cleaners or organic solvents, such as alcohol or acetone, to clean the system because they may damage the exterior surfaces.

Clean the carousel by hand using warm water and, if necessary, mild detergent.

Troubleshooting

Problem	Possible Cause	Corrective Action
No image when scanning	• Power off or cable loose	Check all connections and power.
	• Loss of laser power	Contact technical support.
	• Image display disabled	Enable image display
Intermittent problems scanning	• Loose cable	Check all rear connections.
Scanner fails with probe array inside	• Power failure	Manually extract probe array. Check all connections to scanner. Turn scanner on, restart software.
Carousel does not automatically home	• Check for stuck cartridge • Carousel not seated on D ring • Alignment Pin not engaged in Carousel • Door is open or ajar • Door is open when blue LED is off.	

Problem	Possible Cause	Corrective Action
Carousel does not rotate	• Door is open or ajar • System is warming up, chip in heater • Carousel not seated on D ring • Alignment Pin not engaged in Carousel • Laser in Scanner is warming up. GCOS has Start grayed out in this case	
Carousel misses next chip	• Cartridge UP sensor not working, call technical support.	
Stuck cartridge		See the section entitled *Manually Removing a Lodged Probe Array Cartridge* on page 701
AutoLoader freezes up	• Door is open or ajar	
AutoLoader overheats	• Heater Failure • TE failure • TE hot fans vent blocked	Call technical support. Call technical support.
Autofocus routine fails to conclude		• Try to rescan chip. • Check for salt on chrome border. If still error, call technical support.
The cartridge does not descend into scanner.	• Carousel not seated correctly • Door is open or ajar • Heater is waiting until chip is at temperature.	

AutoLoader Error Messages

The following error messages indicate a serious malfunction of the scanner with AutoLoader. Your probe arrays, or the data generated from them, may be at risk. You should shut down the AutoLoader and remove the carousel.

Do not continue to use the AutoLoader in Automode. Call Affymetrix Technical Support.

However, if the AutoLoader appears to be operating normally, you can continue to use the AutoLoader in Manual Mode. See the section entitled *Scanning a Probe Array in Manual Mode* **on page 690**.

HEATER_LOW	"Warning: The warming chamber temperature is low. Refer to the troubleshooting guide."
COLD_CHAMBER_LOW	"Warning: The cold chamber temperature is low. Refer to the troubleshooting guide."
COOL_HOTSIDE_HIGH	"Warning: The cooler hot-side temperature is high. Refer to the troubleshooting guide." **Note: Before calling technical support, check around the ventilation vents to ensure that nothing is blocking them.**
COLD_CHAMBER_HIGH	"Warning: The cold chamber temperature is high. Refer to the troubleshooting guide." **Note: Before calling technical support, check the AutoLoader door to ensure that it is not open.**
HEATER_HIGH	"Warning: The warming chamber temperature is high. Refer to the troubleshooting guide."

Manually Removing a Lodged Probe Array Cartridge

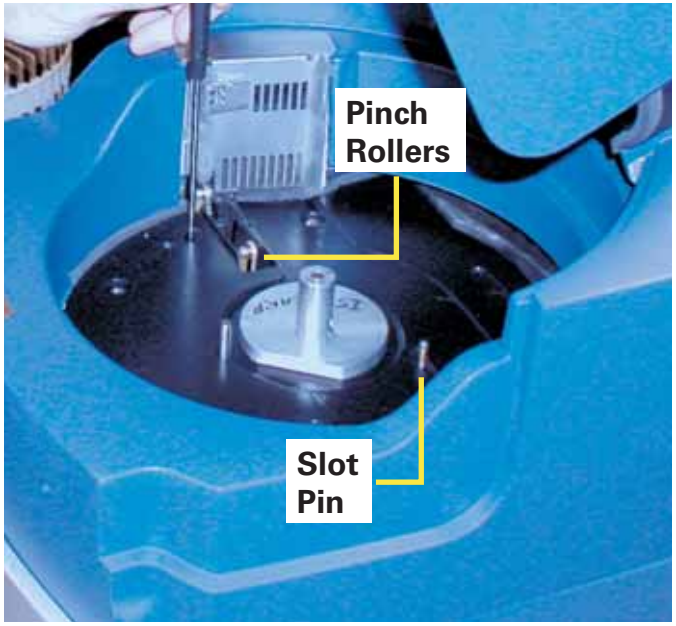
In the event that a probe array becomes lodged in the chip transport mechanism, follow the procedure outlined below.

1. **Turn the AutoLoader off and remove the power cord from the back of the unit.**
2. **Open the AutoLoader door on top of the unit.**
3. **Remove the carousel from the system. (Keep cartridges in carousel at proper temperature while recovering the cartridge still in the scanner).**

4. **Remove the hole plug, which is just in front of the cartridge slot in the base piece of insulation. In the photo to the left, the screwdriver is inserted into this hole.**

5. **Using a standard, flat (-) screwdriver, (13-0257) gently slide it down through the hole making sure not to damage the shaft and spring that are protruding into the hole. When the screwdriver stops, it should be in contact with the scanner Y stage screw. Slowly turn the screwdriver until you feel it engaging the slot on the screw of the scanner Y stage.**
6. **Slowly turn the screw clockwise until it hits a hard stop and cannot turn further. (Do not try to turn it further or use excessive force because it will break the Y stage in the scanner). The Y stage has now ascended to its maximum position.**

7. **Using your fingers, slowly slide the slot pin, which is sticking through the slot in the base piece of insulation, to the right until it stops. You should see the little pinch rollers near the cartridge slot close a little as you do this.**



8. **Insert a 3/16” hex driver (13-0255) into the hole that is located on the front of the AutoLoader housing on the left. You should feel it engage a coupling.**

9. **Turn the hex driver counter clock wise until you see the cartridge appear through its opening. (The cartridge should stay up if you stop turning the hex driver). If you don't see the cartridge after turning the hex driver ten seconds go to step 11.**

10. **Grab and hold the cartridge with your fingers. Using your other hand slowly slide the slot pin (Step 7) back to the left. This should open up the pinch rollers. Pull the cartridge out.**

11. **If you do not see the cartridge after turning the hex driver for 10 seconds, stop.**

12. **Using tool (13-0256) with the hook down and toward the back, slide it vertically down against the front of the cartridge opening, about 1.5 inches. (There is a small groove made for this tool in the middle of the front cartridge guide)**

13. **Pull the top finger grip of the tool toward the front of the unit, and then pull it up while still putting pressure towards the front. The cartridge should come up with the tool. When you see it, grab the cartridge and pull it out of the unit.**

14. **If you cannot get the cartridge out after doing this procedure, call for Affymetrix technical support.**

15. **Put the hole plug back into the hole in the base piece of insulation.**

16. **Plug the scanner back in and turn it on.**

17. **Load the carousel after the scanner boots up.**

18. **If cartridges continue to become lodged in the AutoLoader, you should call technical support.**

GeneChip® Scanner 3000 - AutoLoader Specifications

Item	Parameter	Value
Weight	Shipping	approx 115 pounds (52.2 Kg)
	Free-standing	approx 100 pounds (45.4 Kg)
Dimensions	Width	~13.25 in.
	Depth	~21.25 in.
	Height	~32 in.
Power	See the section entitled *Scanner 3000 Specifications* on page 503.	
Working Environment	Temperature	59°F-85°F (15°C-30°C)
	Humidity	10-90% Non-condensing
	Clearance	2 in. (5 cm) on side, back 12.5 in. on top
	Pollution Degree	2
	Installation Category	II
	Altitude	<2000m
Electrical Supply	See the section entitled *Scanner 3000 Specifications* on page 503.	
Main Supply Voltage Fluctuations	See the section entitled *Scanner 3000 Specifications* on page 503.	

CE Mark Declaration of Conformity

We, Affymetrix, Inc.
4G Crosby Drive,
Bedford, Massachusetts

Declare under sole responsibility that the **Affymetrix® GeneChip® Scanner 3000-AutoLoader** conforms with the relevant provisions of the following standards or other normative documents:

EU EMC Directive 89/336/EEC:

EN 61326:1998		Equipment for Measurement, Control and Laboratory Use, EMC Requirements
	EN 55011:1998	Industrial, scientific and medical (ISM) radio-frequency equipment - Radio disturbance characteristics - Limits and methods of measurement
	EN 61000-3-2:2001	Limits for harmonic current emissions (equipment input current up to and including 16 A per phase)
	EN 61000-3-3:1995	Limitation of voltage changes, voltage fluctuations and flicker in public low-voltage supply systems, for equipment with rated current less than or equal to 16 A per phase and not subject to conditional connection
	EN 61000-4-2:1995	Electrostatic discharge immunity.
	EN 61000-4-3:1995	Radiated, radio frequency, electromagnetic field immunity.
	EN 61000-4-4:1988	Electrical fast transient/burst immunity.
	EN 61000-4-5:1995	Surge immunity.
	EN 61000-4-6:1996	Immunity to conducted disturbances induced by radio frequency fields.
	EN 61000-4-11:1994	Voltage dips, short interruptions, and voltage variations immunity.

EU Low Voltage Directive 73/23/EEC

EN 61010-1:2001	Safety requirements for electrical equipment for measurement, control, and laboratory use -- Part 1: General requirements
EN 60825-1:1994+ A2:2001	Safety of laser products -- Part 1: Equipment classification, requirements and user's guide

Regulatory

This device complies with Part 15 of FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) This device must accept any interference received, including interference that may cause undesired operation.

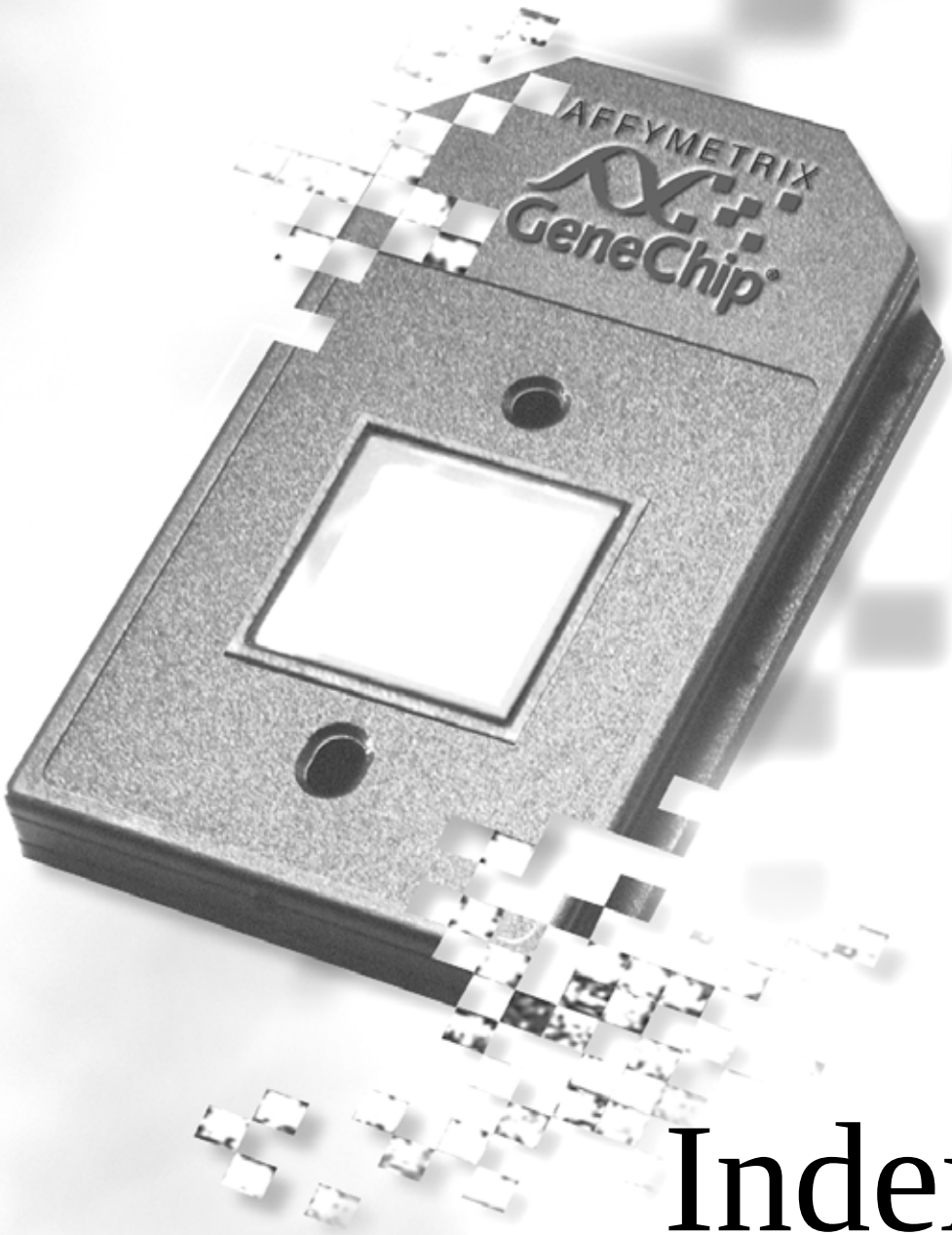
This device complies with FDA performance standards for laser products except for deviations pursuant to Laser Notice No. 50, dated July 26, 2001.

This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulation.

Cet appareil numérique de la classe A respecte toutes les exigences du Règlement sur le matériel broullier du Canada.

Regulatory Agency	Certification
	AL 02 12 39816 004
	92AA
	See the section entitled *GeneChip® Scanner 3000 - AutoLoader Specifications* on page 706.
Class I Laser Device	21 CFR 1040.10 and 1040.11

For Research Use Only. Not for use in diagnostic procedures.



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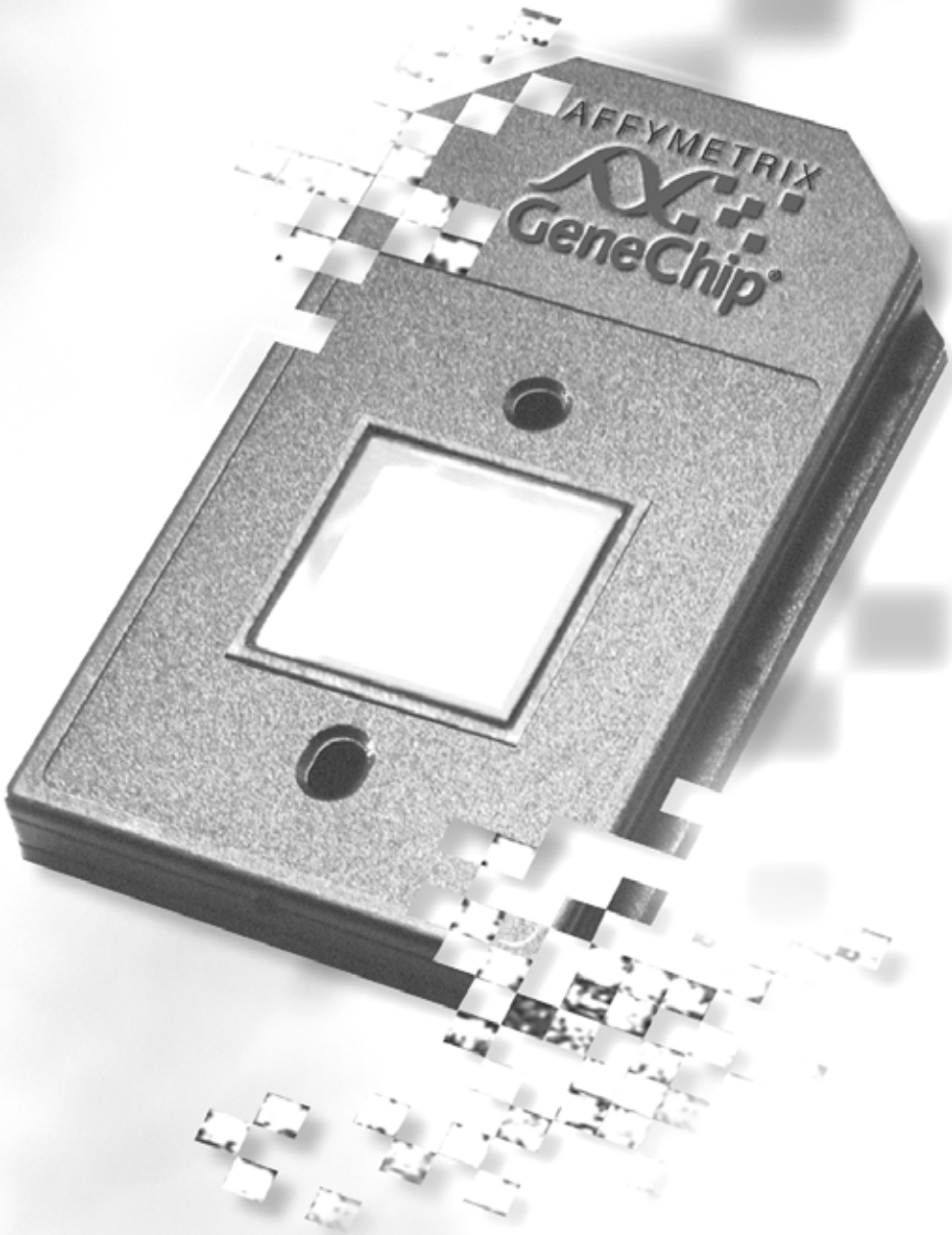

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