Affymetrix®

User's Guide

GeneAtlas® System User's Guide

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P/N 08-0306 Rev. B

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Patents

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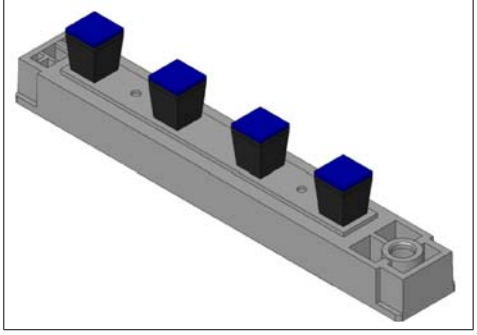
The GeneAtlas® Instrument Overview

Introduction

The GeneAtlas® Instrument is a modular microarray system for processing four arrays in parallel. Each of the four arrays are attached to "pegs" and arranged on a single strip (Figure 1.1). The GeneAtlas System can run about two strips per day. Refer to the Affymetrix website for more information.

Figure 1.1 The GeneAtlas® **System** Four Array Strip



Affymetrix designed the GeneAtlas® Imaging Station and Fluidics Station (Figure 1.2) to assist processing samples. After completing target preparation, you then perform the hybridization, washing and staining, and imaging processes of the workflow on separate modular components, i.e., the Fluidics Station and Imaging Station, which are connected to a single computer workstation as illustrated in Figure 1.3.

Figure 1.2 The GeneAtlas® Imaging Station and Fluidics Station

Figure 1.3 The GeneAtlas® Instrument Connections

The workflow for processing an array strip on the GeneAtlas System is simple and requires limited hands-on time. Table 1.1 illustrates the workflow used in the GeneAtlas System and the components used to perform that workflow. A computer workstation monitors and controls all of the workflow steps.

Table 1.1 GeneAtlas® Instrument Workflow

Step in Workflow	Instrument	Component	Description
Register Samples		Barcode Reader	▪ Scan the array strip barcode.The application enters the names into the computer workstation. ▪ Add target samples to the hybridization tray and insert the array strip into the hybridization tray.
Hybridize Array Strip		Hybridization Station	▪ Scan the array strip barcode. The application automatically populates the naming information. ▪ Place the array strip on the hybridization station and click start on the user-interface. ▪ The computer workstation will track and log the hybridization time for each strip.
Wash and Stain Array Strip		Fluidics Station	▪ Scan the array strip barcode. The application automatically populates the naming information. ▪ Load wash, imaging and hyb trays
Image Array Strip		Imaging Station	▪ Scan the array strip barcode. The application automatically populates the naming information. ▪ Click the **Open Imaging Station** button, load a array strip, and Click the **Close Imaging Station** button. The Imaging Station images the fluorescent labeled array and converts the intensity information into .cel files. These .cel files contain a single signal value for each array feature.

Self-Installation

We have designed the GeneAtlas System for simple set-up and operation. A single computer workstation, which has the pre-installed instrument control software, connects and controls the GeneAtlas Instrument components.

The *GeneAtlas® System Setup and Verification User's Guide* (P/N 08-0311) provides information about the proper placement of the units on a laboratory bench and connecting the components (Fluidics Station, Imaging Station, Monitor, Keyboard, Mouse, and Barcode Reader) to the computer workstation. The guide also provides information for performing the Instrument Verification (IV) to ensure the system is fully operational after power up.

User Documentation

The operation of the GeneAtlas® Instrument requires familiarity with other user documentation. Those manuals that are relevant for you will depend on your system configuration.

- *Site Preparation Guide—GeneAtlas® System and Assays* (P/N 08-0307)
- *GeneChip 3' IVT Express Manual for use with GeneAtlas® System User's Guide* (P/N 702833)
- *GeneAtlas® System User's Guide* (P/N 08-0306)
- *GeneAtlas® System Setup and Verification User's Guide* (P/N 08-0311)
- Provided Hybridization Oven Manual

Safety Information and Warnings

This section deals with safety issues and hazards concerning the Imaging Station present during regular operation.To ensure safe operation of the GeneAtlas® Instrument, read this section completely before operating the instruments.

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.

Safe Operation

- The GeneAtlas® Instrument is intended for indoor, laboratory use in a controlled environment.
- Do not attempt to service the instruments. Any attempt at unauthorized service may result in injury or damage the instrument and/or void the warranty.
- Failure to properly support the instruments may cause serious damage or injury and may void the warranty.
- The instruments must be surrounded by adequate airspace. Slots and openings in the instruments 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.
- Do not open the instrument electrical cabinets. These contains electrical hazards.

WARNING: Users are not allowed to gain access to the interior of the GeneAtlas Instrument through any other openings except that is needed to perform operations related with consumables. Removing the housing may damage the instrument components and result in hazardous exposure to LED light, hazardous voltage, or moving parts. If the protective housing is damaged, users are not allowed to operate the instrument any more.

WARNING: Do not open the instrument mechanical cabinet or stick fingers into the instrument. Moving the unit's axes can cause a risk of pinch or crush hazards! Be aware of the placement of all assemblies before starting a run. Make sure the instrument's enclosure is secure before beginning a run; if it is not, make sure no one is working inside the system. Read, understand, and follow the safety information contained in this manual prior to operating or using this equipment. Pay close attention to all safety labels.

Mechanical Hazards

Do not open the instrument mechanical cabinet or stick fingers into the instruments. Moving the unit's axes can cause a risk of pinch or crush hazards! Be aware of the placement of all assemblies before starting a run. Make sure the instrument's enclosure is secure before beginning a run; if it is not, make sure no one is working inside the system.

Electrical Hazards

Do not use the instruments if you see damaged or frayed electrical cords. Tag and report them as unsafe.

Do not place any liquids or containers holding liquids on or near electrical systems.

Ergonomic Hazards

The workstation has a user interface that may pose ergonomic issues. To avoid fatigue or muscle pain, follow basic precautions including the following:

- Read, understand, and follow your workplace ergonomic recommendations.
- Move computer monitor, keyboard and mouse (user interface) so that it can be used comfortably.
- Take short, regular breaks away from the instruments.
- Make sure the area is well-lit and you are able to see the information on the screen clearly.

Hazards

Table 1.2 summarizes possible hazards.

Table 1.2 GeneAtlas® Instrument Hazards

Hazard	Present?	Description
Chemical	No	
Control	No	Control software
Electrical	Yes	100-240V power
Ergonomic	Yes	User interface
Gas	No	
Mechanical	Yes	Instrument weight (heavy instrument)
Laser	No	
Noise	No	
Temperature	Yes	Hybridization Station and Fluidics Station
Ultrasonic	No	
Vibration	No	

Table 1.2 GeneAtlas® Instrument Hazards (Continued)

Hazard	Present?	Description
E-Fields	No	
H-Fields	No	

IMPORTANT: **If you use the GeneAtlas® Instrument in a manner not specified in this user's guide, you may impair the protection provided by the equipment.**

Electromagnetic Compatibility (EMC)

A good EMC environment is critical to the instrument since large noises may lead to unpredictable results. Please consider the following cautions:

- Keep the instrument away from high dischargeable equipment, such as pacemakers, electric welding equipment, etc.
- Keep the instrument away from frequently starting-up high power consuming equipment, such as refrigerators, centrifuges, etc.
- Keep the instrument away from any strong magnetic field.
- Do not connect many power cables to the junction box to which the instrument is connected.
- Do not plug in or pull out any other equipment to the same junction box while the instrument is running.

GeneAtlas® Instrument Specifications

Table 1.3, Table 1.4 and Table 1.5 list the important instrument specifications.

Table 1.3 The Specifications of the Combined GeneAtlas® Instrument

Item	Parameter	Value
Working Environment (**indoor use only**)	Temperature Range	59 °F to 86 °F (15 °C to 30 °C)
	Relative Humidity Range	10 - 90%
	Pollution Degree	2 environment
	Installation Category	II
	Altitude	<2000m
Shipping and Storage Conditions	Temperature Range	-40 °F to 1 40 °F (-40 °C to 60 °C)
	Relative Humidity Range	10 - 95%
Electrical Supply	Provide voltage, frequency or power rating per unit label. Circuit breaker.	
Main Supply Voltage Fluctuations	Mains supply voltage fluctuations up ±10% of the nominal supply voltage (Transient overvoltages typically present on the mains supply)	

Table 1.4 Specifications of the GeneAtlas Imaging Station

Specifications	Description
Supported Array Formats	Array strip mated with the imaging tray specified in reference drawing 99-027384-01.
Excitation Wavelengths (nm)	530
Autofocus Wavelengths (nm)	▪ 590 nm for Revision B, serial numbers 91000210 - 91001960 ▪ 617 nm for Revision C, serial numbers 91001970 and up
Resolution	2 µm
Sensitivity	0.1flors/µm2
Imaging Time (for a strip of 4 arrays)	<1 hour
Digital Resolution	12 bits
File Format	DAT
Operating System	Windows 7, Windows 2000 (with Service Pack 4), or Windows XP (with Service Pack 2)
Dimensions	15.3"(389 mm) (L) × 6.4" (164 mm) (W) × 11.9" (303 mm) (H)
Noise(dB)	<56
Weight (Kg)	11
Power Supply	Voltage100 - 240 V (± 10%) Voltage Current 6.2 - 2.6 A Line Frequency 50 - 60 Hz

Table 1.5 Specifications of the GeneAtlas Fluidics Station

Specifications	Description
Supported Array Formats	Array strip mated with the scan tray specified in reference drawing 99-027384-01.
Supported Imaging Tray	Specified in reference drawing 99-027384-01.
Supported Hybridization Tray	Peter Joyce, Consumables_DIR_Rev1.doc, Aug 26, 2006, Affymetrix.
Supported Wash A Tray	Peter Joyce, Consumables_DIR_Rev1.doc, Aug 26, 2006, Affymetrix.
Supported Wash B Tray	Peter Joyce, Consumables_DIR_Rev1.doc, Aug 26, 2006, Affymetrix.
Wash stain Time (one array strip)	About 2 hours
Operating System	Windows 7, Windows 2000 (with Service Pack 4), or Windows XP (with Service Pack 2)
Dimensions	16.8" (428 mm) (L) ×16.5" (420 mm) (W) × 14.3" (364 mm) (H)
Noise(dB)	<56
Weight (Kg)	12
Power Supply	Voltage100 - 240 V (± 10%) Voltage Current 6.2 - 2.6 A Line Frequency 50 - 60 Hz

Required Lab Equipment and Supplies

You must have the following reagents, instruments and supplies available in order to perform a run on the GeneAtlas Instrument. Table 1.6 and Table 1.7 list the GeneChip® 3' IVT Express kit and components, required reagents and other supplies.

Table 1.6 Reagents

Material	Source	P/N
GeneChip® 3' IVT Express Kits	Affymetrix	901228 (10 Rxn)* 901229 (30 Rxn)†
GeneAtlas® Hybridization, Wash, and Stain Kit for 3' IVT Arrays containing: *Box 1* of 2 ▫ GeneAtlas® 1X Pre-Hybridization Mix ▫ GeneAtlas® 1.3X Hybridization Mix Solution A ▫ GeneAtlas® 1.3X Hybridization Mix Solution B ▫ Nuclease-free water ▫ GeneAtlas® Stain Cocktail 1 ▫ GeneAtlas® Stain Cocktail 2 ▫ GeneAtlas® Array Holding Buffer *Box 2* of 2 ▫ GeneAtlas® Wash Buffer A ▫ GeneAtlas® Wash Buffer B	Affymetrix	901531 (60 Rxn)
100% ethanol (ACS reagent grade)‡	multiple	

** The 10 reaction GeneChip 3' IVT Express Kit will produce 20 reactions on the GeneAtlas System.*

† The 30 reaction GeneChip 3' IVT Express Kit will produce 60 reactions on the GeneAtlas System.

‡ Or equivalent.

Table 1.7 Lab Equipment and Supplies

Material	Source	P/N
Thermal Cycler with heated Lid (capable of holding 0.2 mL tubes for reaction incubations and with appropriate adaptors to accommodate strip tubes)	multiple	
Vortex Mixer (with flat top adaptor for strip tubes)	multiple	
Microcentrifuge (with an adapter for the PCR strip-tubes or plates supplied with the kit)	multiple	
Magnetic Stand for 96-well plates	Ambion	#AM10050 (96-well Magnetic Stand) or #AM10027 (Magnetic Stand - 96)
Orbital shaker for 96-well plates (e.g., Barnstead/Lab-Line Titer Plate Shaker)	multiple	
Vacuum Centrifuge Concentrator (Optional)	multiple	
Spectrophotometer (e.g., NanoDrop® ND-8000 UV-Vis Spectrophotometer)	NanoDrop Technologies	ND-8000
Reagents and apparatus for preparation and electrophoresis of agarose gels (Optional)	multiple	
Pipette for 0.1 to 2 µL*	Rainin	L-2
Pipette for 2 to 20 µL*	Rainin	L-20
Pipette for 20 to 200 µL*	Rainin	L-200

Table 1.7 Lab Equipment and Supplies (Continued)

Material	Source	P/N
Pipette for 100 to 1000 µL*	Rainin	L-1000
Sterile-barrier, RNase-free Pipette Tips	multiple	
Bioanalyzer (optional)	Agilent	
Non-stick RNase-free microfuge tubes, 0.5 mL	Ambion	N12350
Non-stick RNase-free microfuge tubes, 1.5 mL	Ambion	12450
Network Cable	multiple	

* *Or equivalent.*

Technical Support

When to Contact Technical Support

Under any of the following conditions, unplug the instrument from the power source and contact Affymetrix Technical Support:

- when the power cord is damaged or frayed;
- if any liquid has penetrated the instrument;
- if, after service or calibration, the instrument does not perform to the specifications stated in Table 1.3, Table 1.4 and Table 1.5.
- If the instrument must be returned for repair, call Affymetrix Technical Support.

IMPORTANT: **Make sure you have the model and serial number.**

Affymetrix provides technical support to all licensed users via phone or E-mail. Contact information is listed below.

Affymetrix, Inc.
3420 Central Expressway
Santa Clara, CA 95051
USA

E-mail: support@affymetrix.com
Tel: 1-888-362-2447 (1-888-DNA-CHIP)
Fax: 1-408-731-5441

Affymetrix UK Ltd.,
Voyager, Mercury Park,
Wycombe Lane, Wooburn Green,
High Wycombe HP10 0HH
United Kingdom

E-mail: saleseurope@affymetrix.com
E-mail: supporteurope@affymetrix.com
UK and Others Tel: +44 (0) 1628 552550
France Tel: 0800919505
Germany Tel: 01803001334
Fax: +44 (0) 1628 552585

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ORIX Hamamatsucho Bldg, 7F
1-24-8 Hamamatsucho, Minato-ku
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Regulatory and Conformity

GeneAtlas® Instrument Compliance

We

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declare under sole responsibility that the Affymetrix® GeneAtlas® Instrument and associated Workstation with software, is manufactured in conformity with the regulations and certifications stated in this section using U.S. and Non-U.S. components.

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 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 Approval

This device has been approved by the following regulatory agencies (Table 1.8).

Table 1.8 Regulatory Approval

Regulatory Agency	Certification
CE	EU EMC Directive 2008/108/EC EU Low Voltage Directive 73/23/EEC
TÜV Rheinland of North America, Inc. C US	IEC 61010-1 CSA C22.1010.1:1992 (Canada) UL 61010A-1:2002 (USA) EN 61010-1:2001 (EU) Mechanical Safety: EN 1050:1996 IEC 60825-1:1993 +A1:1997 +A2:2001 EN60825-1:1994 +A1:2002 +A2:2001 for a Class 1 LED product. Product Safety for "Electrical Equipment for Measurement, Control, and Laboratory Use", Pollution Degree 2, Over-voltage Category II: North American standards harmonized to IEC 61010-1: CAN/CSA-C22.2 No.61010-1 :2004 (Canada) UL 61010-1 (USA) Low Voltage Directive 73/23/EEC (EU) EN 61010-1:2001, General requirements EN 61010-2-010:1994+A1, Requirements for laboratory equipment for the heating of materials (as set forth in the Design Input Requirements document) Electromagnetic Conformity for "Industrial, Scientific and Medical" (ISM) equipment, Group I, Class A, industrial locations: ICES-003, Industry Canada, Interference-Causing Equipment Standard, Digital Apparatus, Class A (Canada) FCC Part 15 Radio Frequency Emissions for Class A Equipment (USA) EMC Directive 89/336/EEC (EU) EN 61326:1997/A2:2001, General EMC Requirements EN 55011:1998, Radio Frequency Emissions EN 61000-3-2:2000, Harmonic Current Emissions EN 61000-3-3:1995, Voltage Fluctuations and Flicker
	Compliant with directive 2002/96/EC (WEEE) 371123740 (WEEE German Registration) WEEE Registration–France
	Complies with requirements of Radio communications (Electromagnetic Compatibility) Standard 2008

CE Mark Declaration of Conformity

The Affymetrix® GeneAtlas® Instrument conforms with the relevant provisions of the following standard(s) and/or other normative document(s):

GeneAtlas Imaging Station	EMC Directive 89/336/EEC Low Voltage Directive 73/23/EEC
	ICES-003, Industry Canada, Interference-Causing Equipment Standard, Digital Apparatus, Class A (Canada) FCC Part 15 Radio Frequency Emissions for Class A Equipment (USA) CAN/CSA-C22.2 No. 61010.1-04 (Canada) as harmonized to IEC 61010-1 UL 61010-1 (USA) as harmonized to IEC 61010-1
GeneAtlas Fluidics Station	EMC Directive 89/336/EEC Low Voltage Directive 73/23/EEC
	ICES-003, Industry Canada, Interference-Causing Equipment Standard, Digital Apparatus, Class A (Canada) FCC Part 15 Radio Frequency Emissions for Class A Equipment (USA) CAN/CSA-C22.2 No. 61010.1-04 (Canada) as harmonized to IEC 61010-1 UL 61010-1 (USA) as harmonized to IEC 61010-1
GeneAtlas Hybridization Station	EMC Directive 89/336/EEC Low Voltage Directive 73/23/EEC UL 61010-1:2004 R7.05 (USA) CAN/CSA-C22.2 No. 61010.1:2004 (Canada)
	ICES-003, Industry Canada, Interference-Causing Equipment Standard, Digital Apparatus, Class A (Canada) FCC Part 15 Radio Frequency Emissions for Class A Equipment (USA) CAN/CSA-C22.2 No. 61010.1-04 (Canada) as harmonized to IEC 61010-1 UL 61010-1 (USA) as harmonized to IEC 61010-1

Getting Started With the GeneAtlas® System

The GeneAtlas System provides tools for processing arrays and extracting the intensity data for use by the probe level analysis software.

To fully use the capabilities of the GeneAtlas System, you need to understand:

- The array processing workflow that the GeneAtlas components perform
- The types of files that the GeneAtlas System produces and uses
- The structures that the GeneAtlas System uses to organize the resulting data

This chapter introduces those concepts in:

- *Array Processing Workflow* on page 14
- *File Types in the GeneAtlas® System* on page 16
- *Data Organization in the GeneAtlas® System* on page 18

This chapter also includes an introduction to the GeneAtlas Instrument Control user interface (see *Introduction to the Software Interface* on page 18)

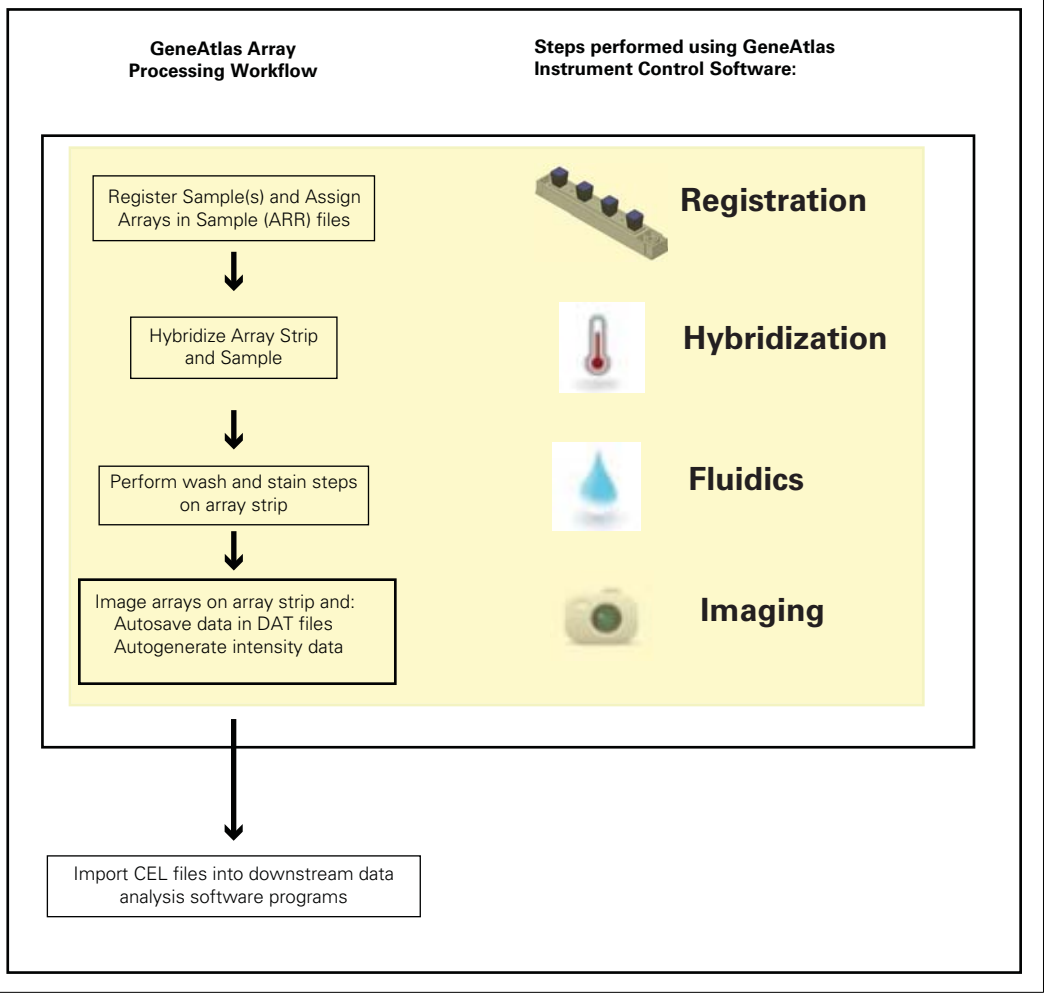
NOTE: See the ***Affymetrix® GeneAtlas® Setup and Verification User's Guide*** for information on setting up and running the verification for the GeneAtlas system.

NOTE: Before running the GeneAtlas System for a particular GeneChip Array, you must have the library files for that array type installed on your computer. For more information, see ***Download Library Files* on page 82**.

Array Processing Workflow

The GeneAtlas Systemis used to process the arrays used in your experiment. The recommended workflow for array strips (Figure 2.1) enables you to include data about the sample and experiment and to easily track the processing steps for the array strip.

Figure 2.1 Array Strip Processing Workflow in the GeneAtlas System



In the recommended array processing workflow for GeneAtlas Array strips, you create sample files for all the arrays on the strip as the first step. You then perform hybridization, wash and stain, and imaging on the strip using the GeneAtlas Fluidics Station, the GeneAtlas Imaging Station, and the GeneAtlas Instrument Control Software. After that, GeneAtlas aligns a grid on the DAT files and computes the Cell Intensity data (CEL) file. The CEL files can then be used for downstream data analysis.

The workflow steps are described in more detail in the following sections:

- *Registering Samples and Arrays*, below
- *Hybridizing Arrays and Samples* on page 15
- *Performing Fluidics* on page 15
- *Running Imager* on page 15
- *Tracking Gridding and CEL file Generation* on page 15

The GeneAtlas Sample and data files that are created during the workflow are described in more detail in *File Types in the GeneAtlas® System* on page 16.

Registering Samples and Arrays

In the GeneAtlas System, the Sample file is the beginning of the data chain for a given experiment. The sample information is stored in a Sample file with an ARR extension. The arrays used in analysis and data files produced by analysis are linked to this Sample File.

The information about the sample and experiment are collected as attributes. The attributes are described in more detail below.

The links between the sample and data files and the GeneAtlas tools used to generate the sample files are described in more detail in *File Types in the GeneAtlas® System* on page 16.

Template and User Attributes

There are two types of sample attributes in the GeneAtlas System:

Template Attributes

A template in the GeneAtlas System is a list of attributes that can be assigned to a Sample file.

An "Express" template is provided with the GeneAtlas application. This template contains a set of attributes that might be useful while analyzing CEL files using the Partek Express Software. You can assign this template to sample files during registration. This automatically adds all the attributes in the template to the Sample file. You can then enter values for each attribute. This allows you to standardize the attributes that are assigned to samples.

User Attributes

User attributes are created on the fly during the registration of a sample and array. This allows you to create a quick note for a particular sample file.

User attributes are not listed in a template; they have usually been added to a specific sample file. They can be used in filtering and search operations, just like the template attributes.

Hybridizing Arrays and Samples

For Array strips, the hybridization is performed in the hybridization station. The GeneAtlas Instrument Control software provides a timer that informs you when the array strip should be removed. See Chapter 4, *Hybridization* on page 35.

Performing Fluidics

The arrays on the Array strip are washed and stained using the GeneAtlas Fluidics Station, controlled using the GeneAtlas Instrument Control software. The software and its use are described in Chapter 5, *Fluidics (Wash and Stain)* on page 43.

Running Imager

The arrays on the Array strip are imaged using the GeneAtlas Imaging Station and the GeneAtlas Instrument Control software, as described in Chapter 6, *Imaging* on page 63.

Tracking Gridding and CEL file Generation

After the array has been imaged, the GeneAtlas system:

- Aligns a grid on the Image (DAT) file to identify the probe cells.
- Computes the probe cell intensity data for the array and creates a CEL file.

The Image Viewer enables you to manually correct gridding problems, if necessary. The Viewer and its use are described in *Using the Viewer* on page 87.

File Types in the GeneAtlas® System

Different types of information are collected by the GeneAtlas System in different types of files:

- Information about the sample and experiment are collected in Sample files (see *Sample Files*, below)
- Probe array data generated during imaging and processing are collected in Data files of various types (see *Data Files* on page 16)
- Audit and Log files contain information about array processing and other processes (see *Other File Types* on page 17)

Sample Files

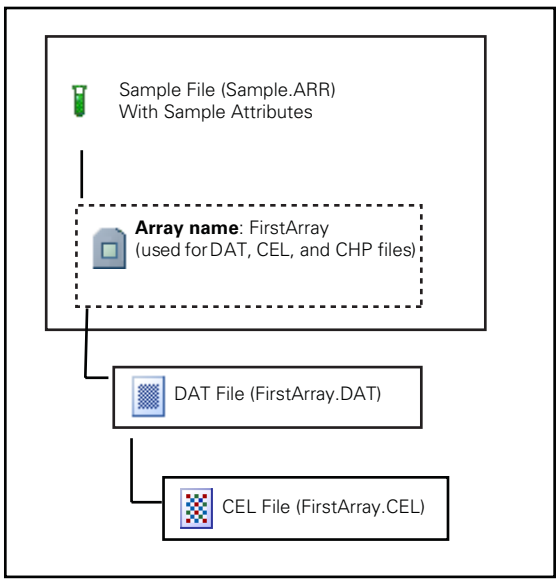
The Sample (.ARR) file (Figure 2.2) collects two types of information:

- Sample Attributes: information that you can use to interpret the experimental data. It can include information about the sample itself, the experimental conditions, or other information you may find useful.

  Some attributes can be used by the probe level analysis software during analysis. You can use templates to manage the attributes used for a particular experiment (see *Template and User Attributes* on page 15 for more information).
- Array Information: Information about the array used with the sample.

  Each array is assigned an array name during registration. The array name is used to identify the DAT, CEL, and CHP data files that are generated during analysis.

Figure 2.2 Sample File with associated arrays and data files



Data Files

A set of data files is produced for each array in the Sample file.

The data files include:

- *Image (DAT) file*, below
- *Intensity (CEL) Data Files* on page 17

Image (DAT) file

The DAT file contains pixel intensity values collected from an Affymetrix Imaging Station, along with the gridding information used during feature extraction.

When a DAT file is regridded, the existing CEL file is over-written. New DAT files and CEL files are created only when an array is re-imaged.

Intensity (CEL) Data Files

The CEL file stores the results of the intensity calculations on the pixel values of the DAT file.

Other File Types

Audit and Log files track the tasks performed by different software components.

Audit Files

An Audit file is an XML file that tracks the processing of each physical array processed by the GeneAtlas System. An Audit file is produced for each physical array and tracks all the processing steps that were performed on the array, including multiple imagings and regridding.

The audit file has the same root name as the physical array.

Log Files

Log files are produced by different GeneAtlas components. The logs provide a record of the tasks performed by different components, such as the migration tools and the installer.

These log files may provide useful information for troubleshooting problems.

The different log files include:

Systemlog.XML	XML file with system information.
UserSettings.XML	XML file with software configuration settings.
fluidics.log	Text file with info on Fluidics Station use
Imager.log	Text file with info on Imaging Station Control use
GeneAtlas_LibFileImporter.log (with date and time code)	Text file with info on use of the Library File Importer.
FS.log (with date and time code)	Text file with information on the Fluidics Script installation.

The log files can be found in the C:\Command Console\Logs\ folder and can be viewed with a text editor or web browser.

Log files for the GeneAtlas Instrument control processes are placed in subdirectories of the C:\Command_Console\Logs\ folder.

You can place relevant log files into a zip folder that you can then send to Affymetrix customer support for troubleshooting (see *Collect Logs* on page 84).

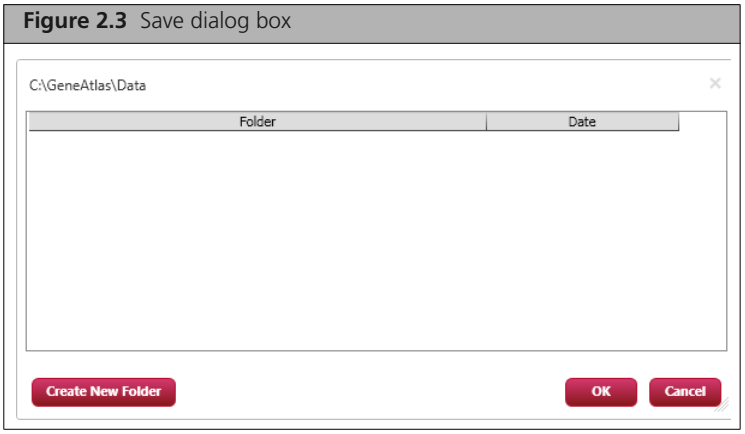
Data Organization in the GeneAtlas® System

To use the GeneAtlas System, you need to understand the structures the software provides for organizing your data during and after generation.

GeneAtlas data is placed in the C:\GeneAtlas\Data folder.

You can create subfolders in the C:\GeneAtlas\Data folder during sample registration, using the **Create New Folder** feature in the Save dialog box.

Figure 2.3 Save dialog box



Introduction to the Software Interface

This section describes basic features of the GeneAtlas Instrument Control Software, including:

- How to start the software
- Parts of the screen
- Basic navigation features.

To start the software:

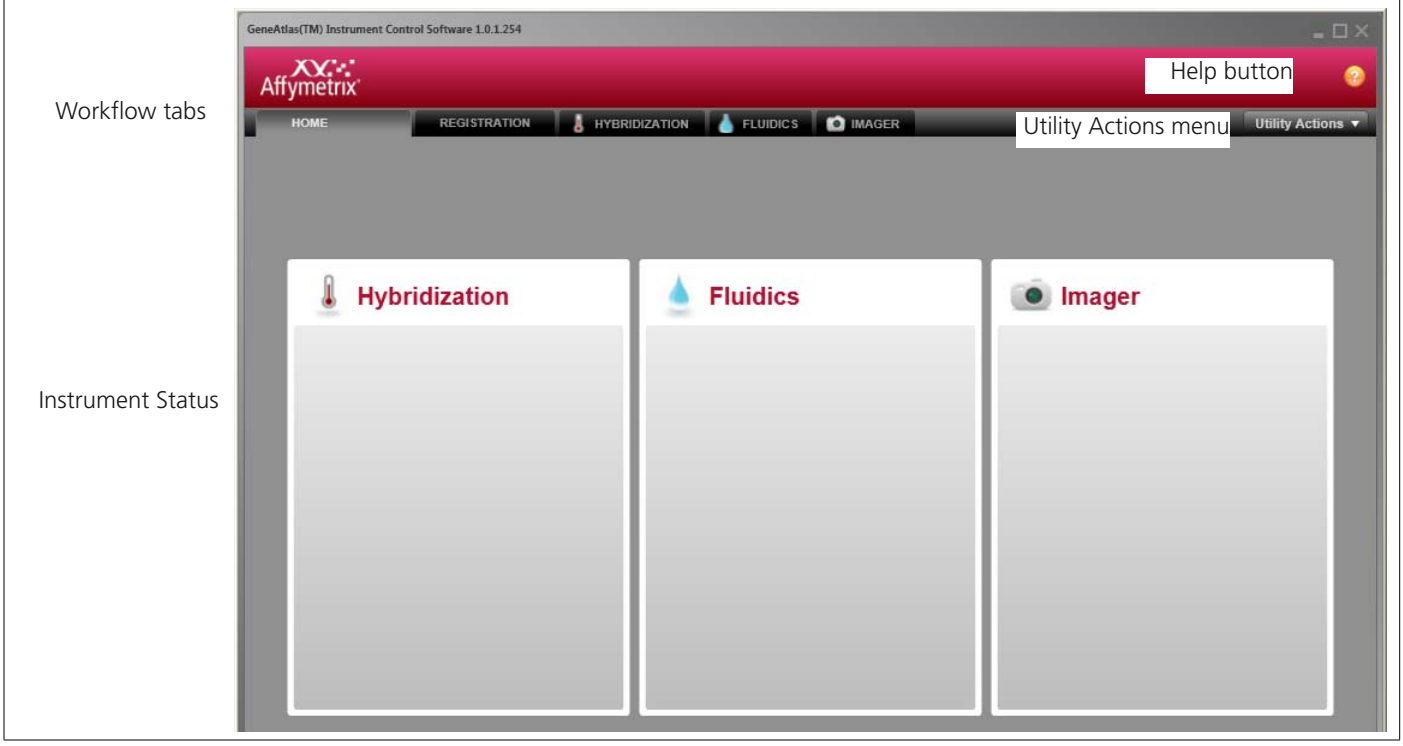
- Click the GeneAtlas Instrument Control software icon on the desktop.

  The software opens to the home tab, described below.

The Home Page

The Home tab (Figure 2.4) provides an overview of the status of the different instruments.

Figure 2.4 Home Page when it opens



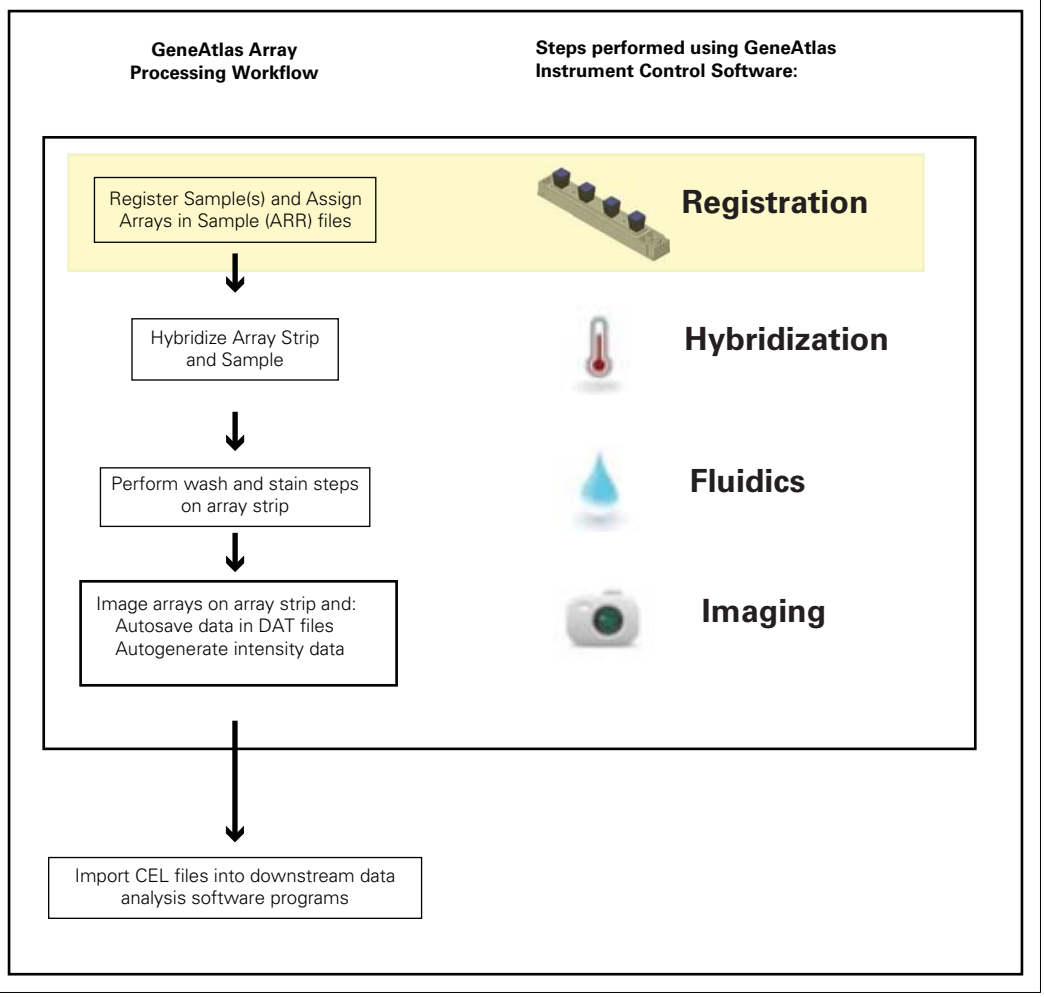
The Home tab has the following components:

Workflow tabs	Allows you to switch between: • Registration tab (see Chapter 3, *Registering Samples* on page 21) • Hybridization tab (see Chapter 4, *Hybridization* on page 35) • Fluidics tab (see Chapter 5, *Fluidics (Wash and Stain)* on page 43) • Imaging tab (see Chapter 6, *Imaging* on page 63)
Instrument Status	Displays current status of instruments
Help button	Click to display help
Utility Actions menu	Used to select utilities (see Chapter 7, *GeneAtlas® Utilities* on page 73)

Registering Samples

Each sample on an array strip must be registered in the GeneAtlas® software prior to processing in the GeneAtlas® System. The registration process creates a sample file (ARR) for each sample (Figure 3.1).

Figure 3.1 Array Processing Workflow



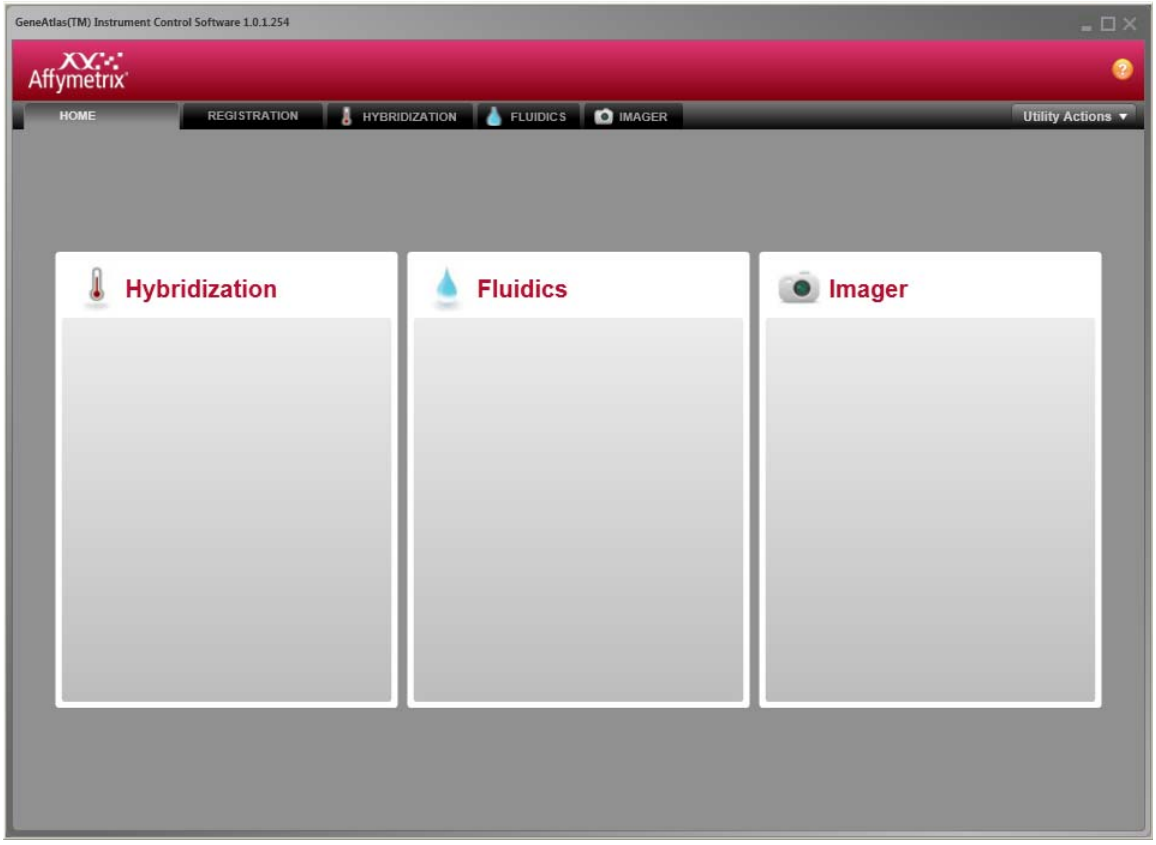
This chapter describes:

- *Sample Registration*
- *Using Templates* on page 28
- *Importing Sample Data* on page 29
- *Editing Sample Files* on page 31

Sample Registration

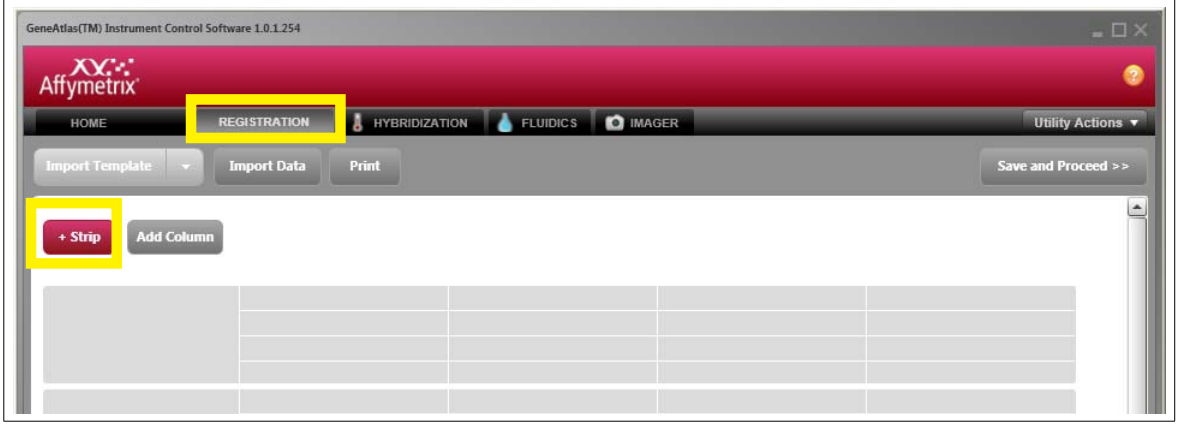
1. Click **Start** → **Programs** → **Affymetrix** → **GeneAtlas** or click on the GeneAtlas shortcut on the desktop to launch the GeneAtlas software.
   The GeneAtlas Home window appears (Figure 3.2).

Figure 3.2 Home window



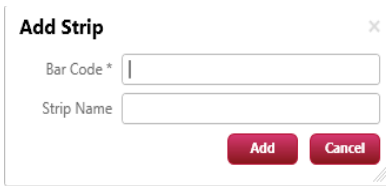
2. Click the **Registration** tab (Figure 3.3).

Figure 3.3 Registration window



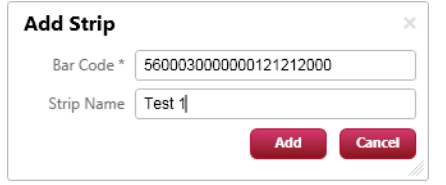
3. Click the + **Strip** button: + Strip.
The Add Strip dialog box appears (Figure 3.4).

Figure 3.4 Add Strip dialog box



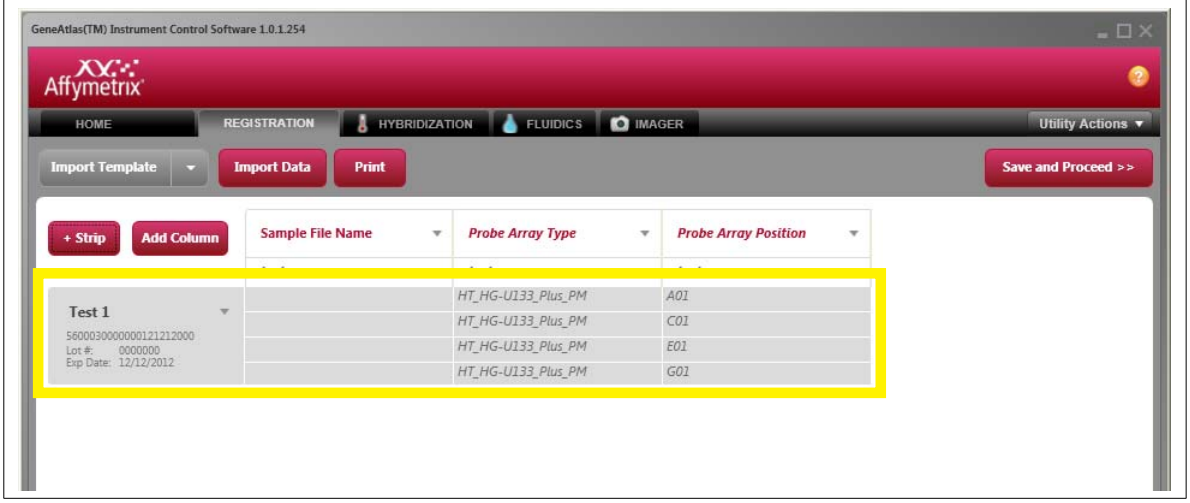
4. Enter or scan the Array Strip bar code and enter a strip name (optional) (Figure 3.5).

Figure 3.5 Add Strip dialog box with Bar Code and Name entered



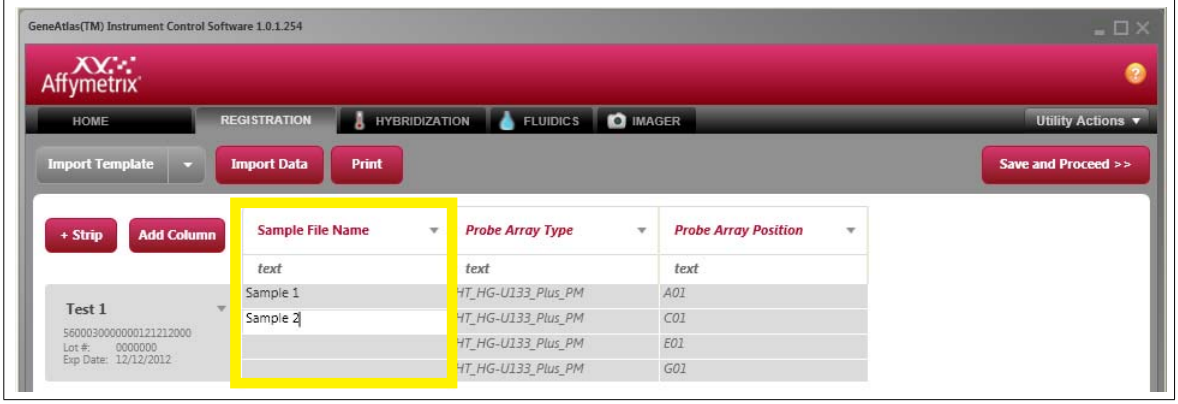
5. Click **Add**
The Array Strip is added to the data grid in the Registration window (Figure 3.6).

Figure 3.6 Registration window, array strip added to the data grid



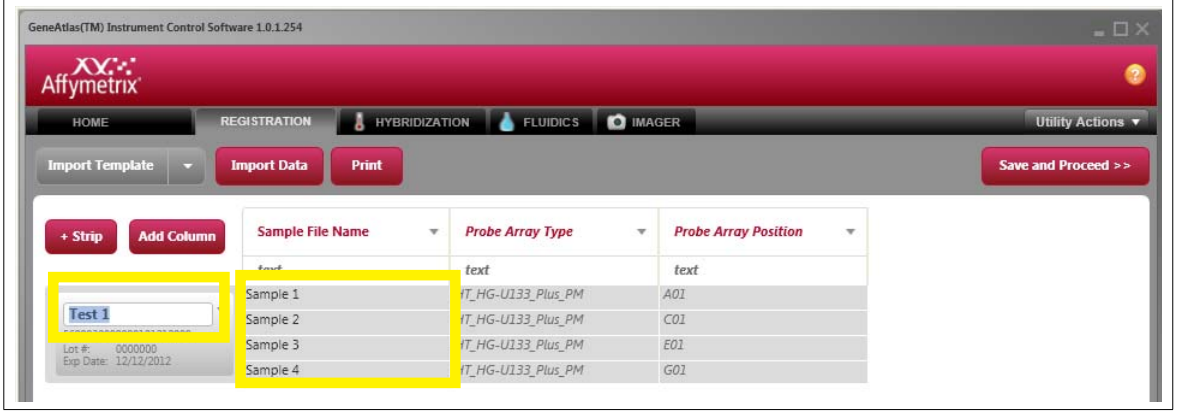
6. Click a box under the **Sample File Name** column, enter the sample name, and press the **Enter** key. Enter a unique name for each of the four samples on the Array Strip (Figure 3.7).

Figure 3.7 Entering Sample File Names



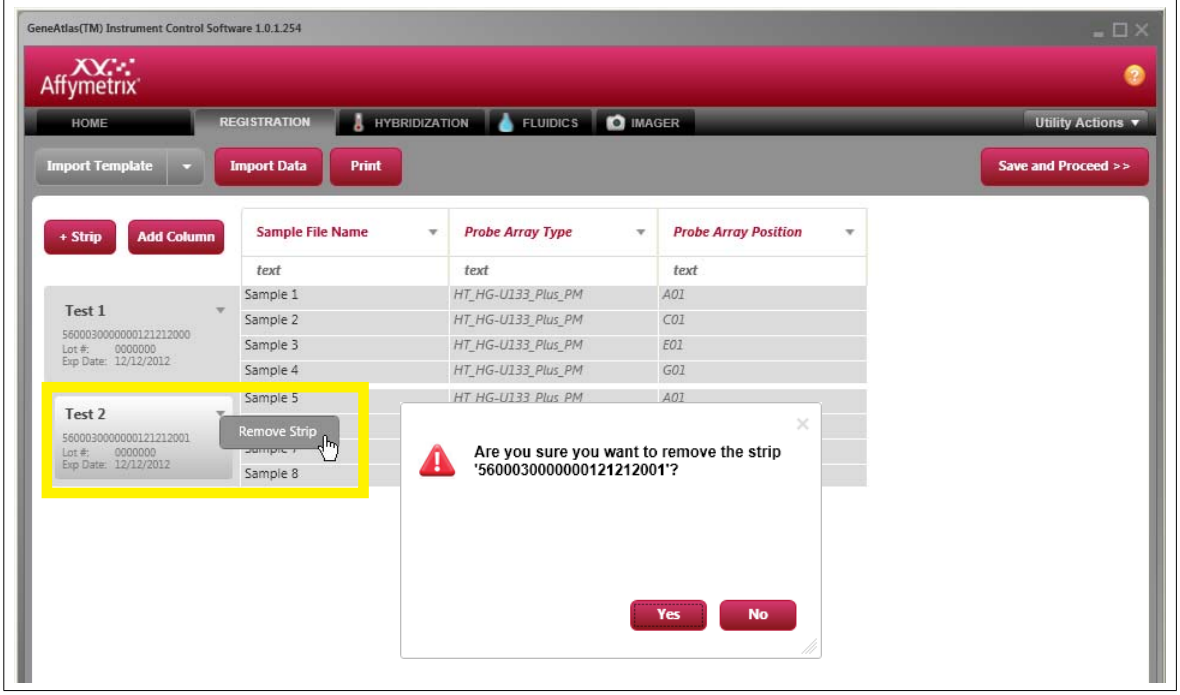
7. To edit the Array Strip name or a sample name, select the name and enter a new name (Figure 3.8).

Figure 3.8 Editing an Array Strip name or a sample name



8. To remove an Array Strip from the data grid:
   A. Click the Array Strip name and select **Remove Strip** from the drop-down list (Figure 3.9).
   B. In the prompt that appears, click **Yes**.

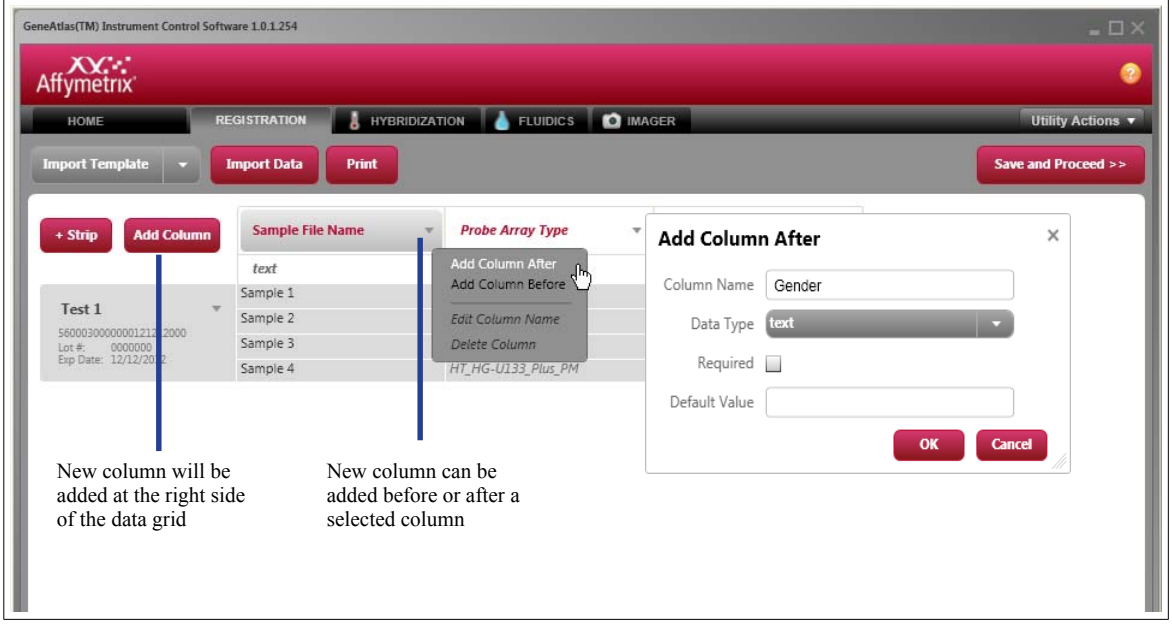
Figure 3.9 Removing a strip from the data grid



9. To add a sample attribute to the data grid:

   A. Click the **Add Column** button: Add Column. Alternately, click a column header and select **Add Column After** or **Add Column Before** from the drop-down list (Figure 3.10). The Add Column dialog box appears.

Figure 3.10 Adding columns to the data grid

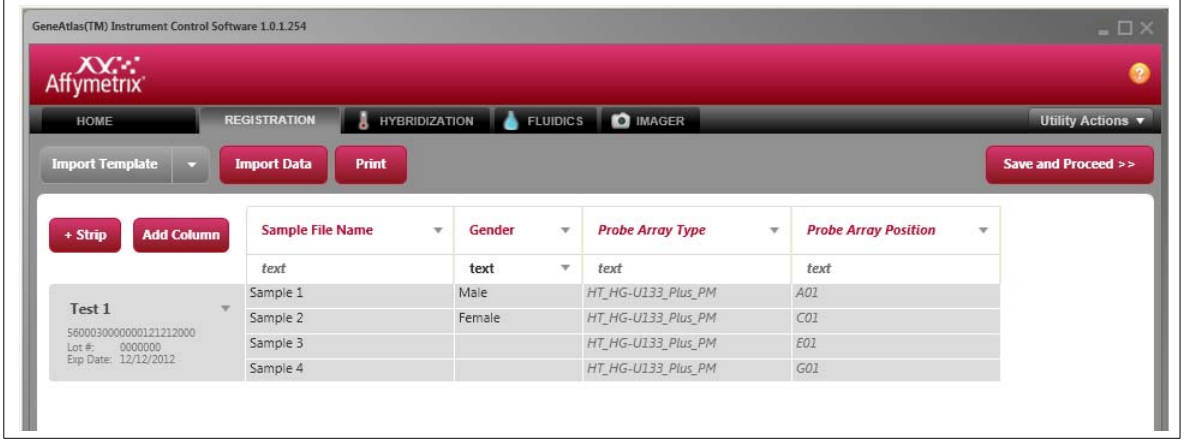


   B. Enter the column name and select a data type (text, number, or date) from the drop-down list.

   C. If an attribute value is required, put a check mark next to **Required**. If applicable, enter a default attribute value.

D. Click **OK**.
The new column appears in the data grid (Figure 3.11).

Figure 3.11 New attribute column



E. In the data grid, enter the sample attribute values.

NOTE: You can import sample data to populate the data grid (see page 29). Some attribute values can be edited in the sample file (ARR) (see page 30).

10. When sample registration is complete, click the **Save and Proceed** button: Save and Proceed >>.
The Save dialog box appears (Figure 3.12).

Figure 3.12 Save dialog box

11. You can save the data in the default folder or create a new folder for the data

A. Click Create New Folder.
The Create New Folder dialog box opens (Figure 3.13).

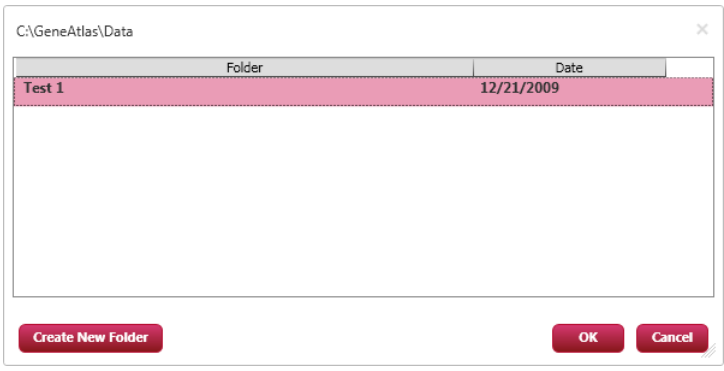
Figure 3.13 Create New Folder dialog box

B. Enter a name for the new folder.

C. Click **Create**.

D. Select a folder in which to save your data (Figure 3.14).

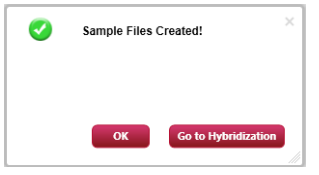
Figure 3.14 Selecting a folder



12. Click **OK** in the Save dialog box.

Your sample files (ARR) are saved to the selected folder and a confirmation message appears (Figure 3.15).

Figure 3.15 Confirmation Message

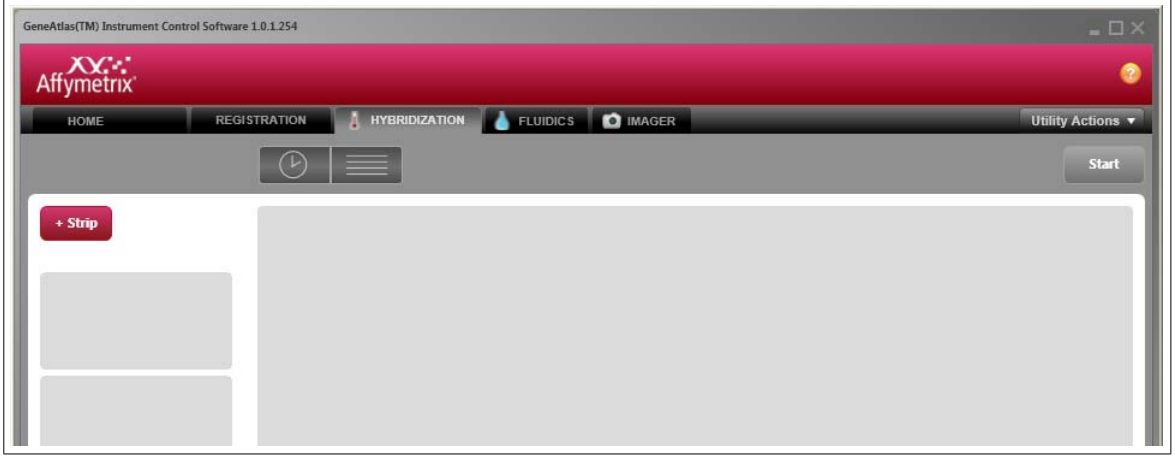


13. Click **OK**; or

Click **Go to Hybridization** to proceed to the Hybridization tab (Figure 3.16).

NOTE: You may enter up to four array strips simultaneously during the registration process.

Figure 3.16 Hybridization window



Using Templates

In the data grid, the default columns are File Name, Probe Array Type, and Probe ArrayPosition. Templates provide additional column headers. A template can also be applied after the sample files are created (for more details, see *Editing Sample Files* on page 31).

The Express template is provided with the GeneAtlas application. This template contains a set of attributes that might be useful while analyzing CEL files using the Partek Express Software. You can assign this template to sample files during registration. This automatically adds all the attributes in the template to the Sample file. You can then enter values for each attribute. This allows you to standardize the attributes that are assigned to samples.

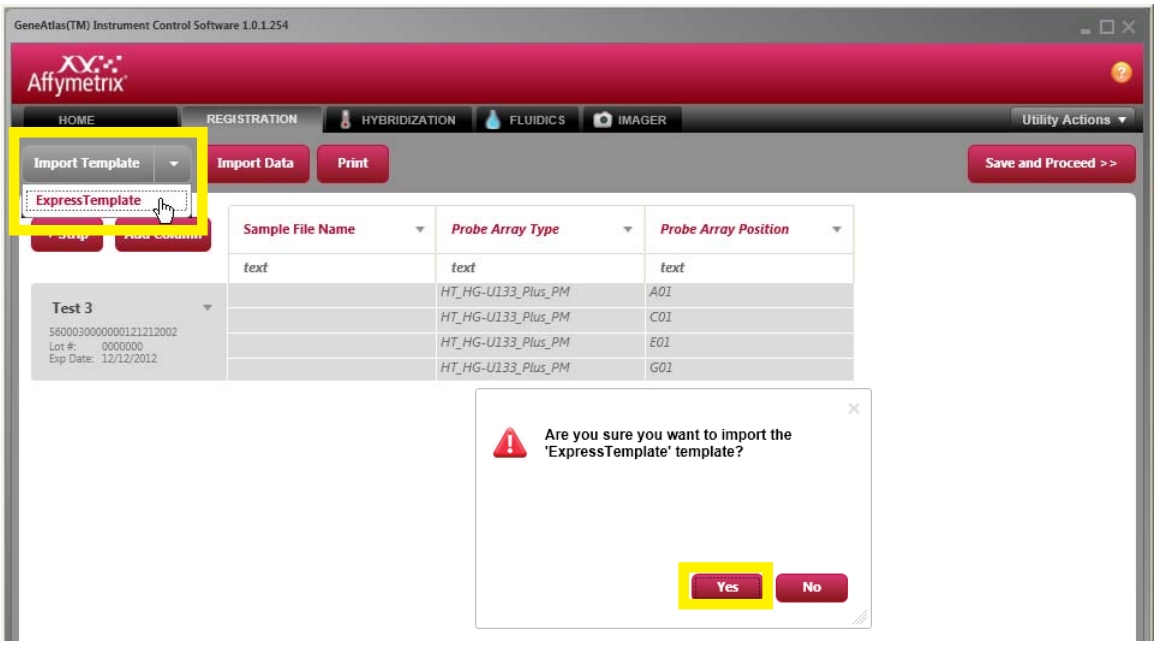
The attributes are:

- Subject ID
- Gender
- Age
- Disease State
- Treatment
- Dose
- Time
- Tissue
- RNA extraction date
- RNA extraction method
- Sample prep date
- Sample prepped by
- Sample source

Importing a Template

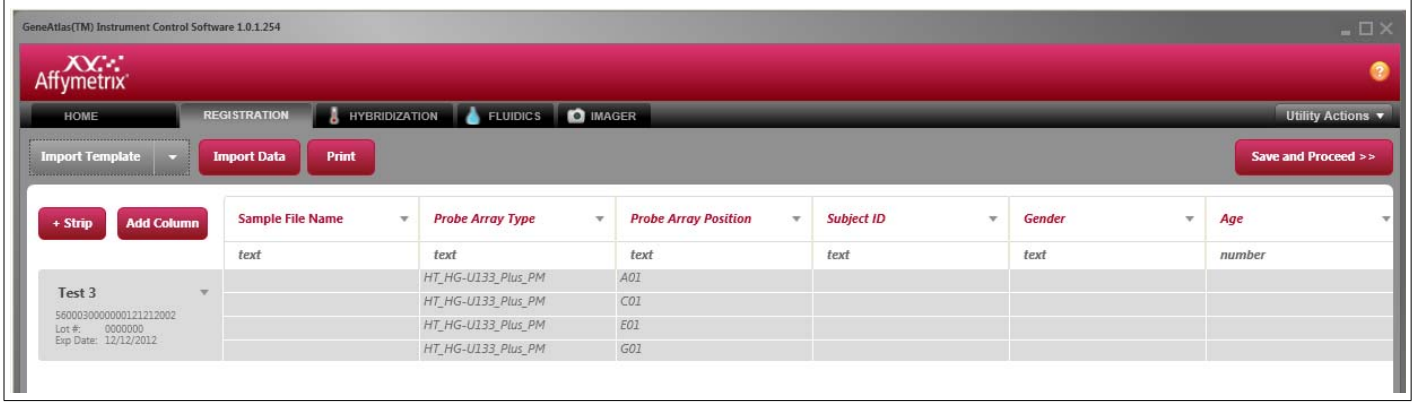
1. In the Registration window, add an Array Strip(s) to the data grid (maximum of four strips). (For details on adding a strip, see **Step 3 to Step 4 on page 23.**
2. Click **Import Template** and select a template from the drop-down list.
3. Click **Yes** in the confirmation message that appears (Figure 3.17).

Figure 3.17 Selecting a template



The template columns appear in the data grid (Figure 3.18).

Figure 3.18 Data grid with template attributes



NOTE: Follow Step 9 on page 25 to add a user-specified column header to the data grid.

Importing Sample Data

You can import sample data to the data grid for more convenient sample registration.

1. In Microsoft® Excel®, create a data file (.xls) that has the column "Sample File Name" in addition to any attributes you want to add. The Probe Array Type and Probe Array Position columns do not need to be included in the data file. Figure 3.19 shows an example data file.

IMPORTANT: If you are using Excel 2007, be sure to save the file in Excel 1997-2003 Compatibility mode (with an .xls extension), rather than Excel 2007 mode (with an .xlsx extension).

Figure 3.19 Example sample data file

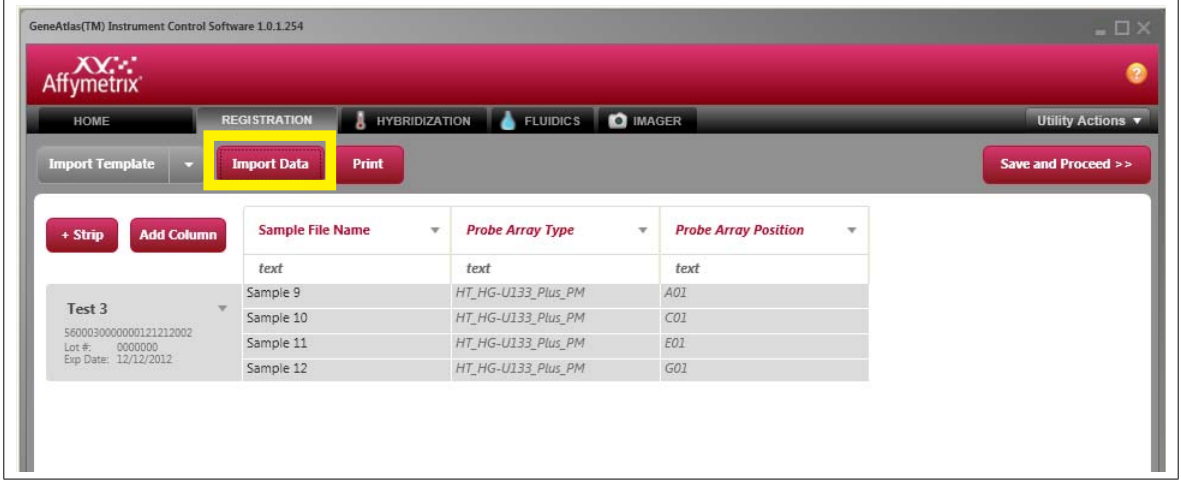
	A	B	C	D	E	F	G
1	Sample File Name	Age	Gender	Sample Type	Tissue	Date of sample prep	
2	Sample 9	25	Male	RNA	Blood	12/15/2009	
3	Sample 10	14	Male	RNA	Kidney	12/8/2009	
4	Sample 11	54	Male	RNA	Lung	11/28/2009	
5	Sample 12	36	Female	RNA	Brain	12/5/2009	
6							
7							

2. In the Registration window, add an Array Strip(s) to the data grid (maximum of four strips). (For details on adding a strip, see **Step 3 to Step 4 on page 23.**
3. Enter the sample file names.

NOTE: If a sample file name in the data grid is not found in the data file (.xls) selected for import, no data will be imported for the sample.

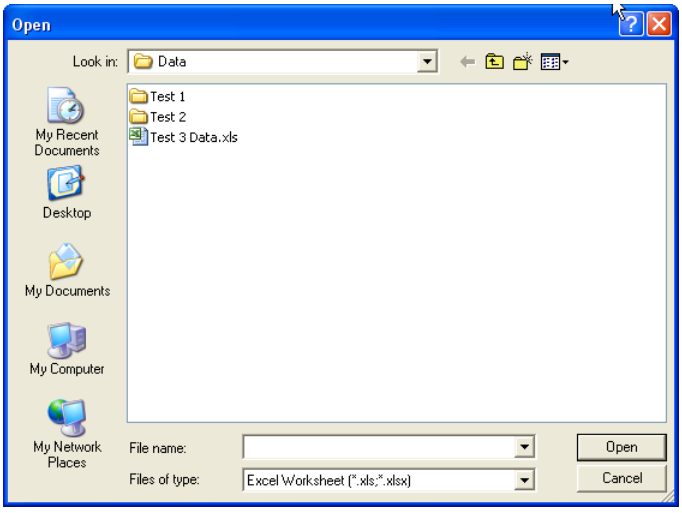
4. Click the **Import Data** button: Import Data.

Figure 3.20



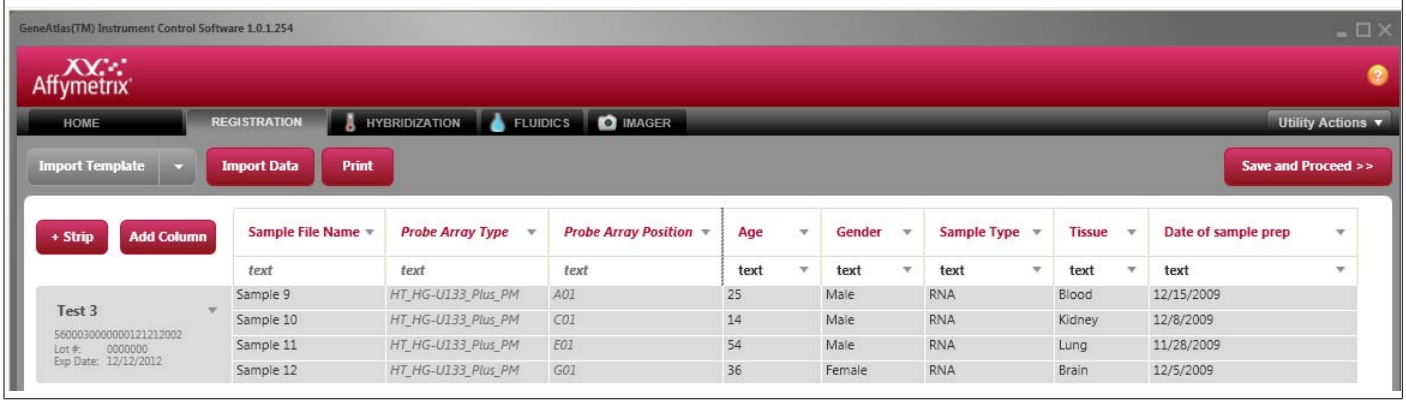
The Open dialog box appears (Figure 3.21).

Figure 3.21 Open dialog box



5. Select the data file (.xls), and click **Open**.
   The sample information from the data file appears in the data grid (Figure 3.22).

Figure 3.22 Imported data displayed



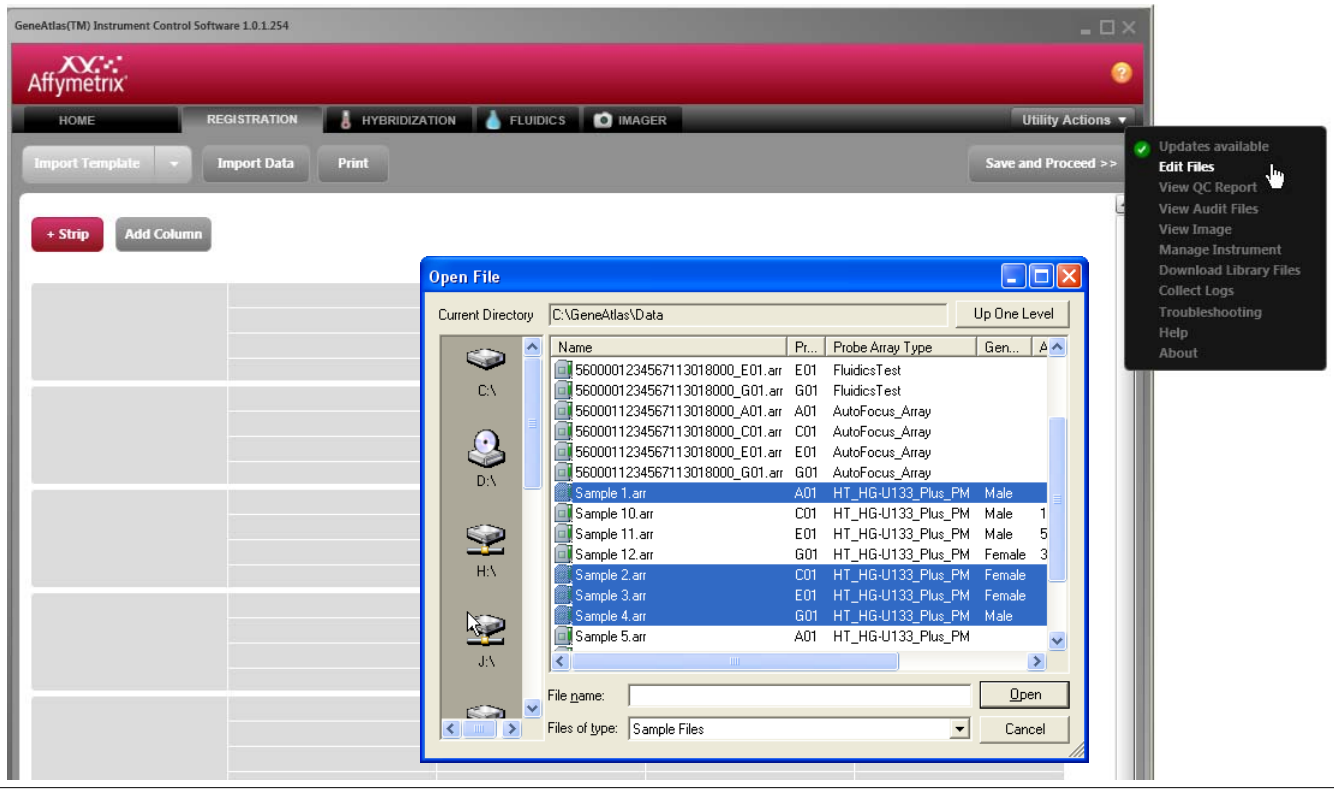
Editing Sample Files

Sample files (ARR) can be edited. You can:

- Import a template
- Edit sample data (not all types of data can be modified)
- Import data
- Add user-specified columns to the data grid

1. To open a sample file(s), select **Edit Files** from the Utilities drop-down list.

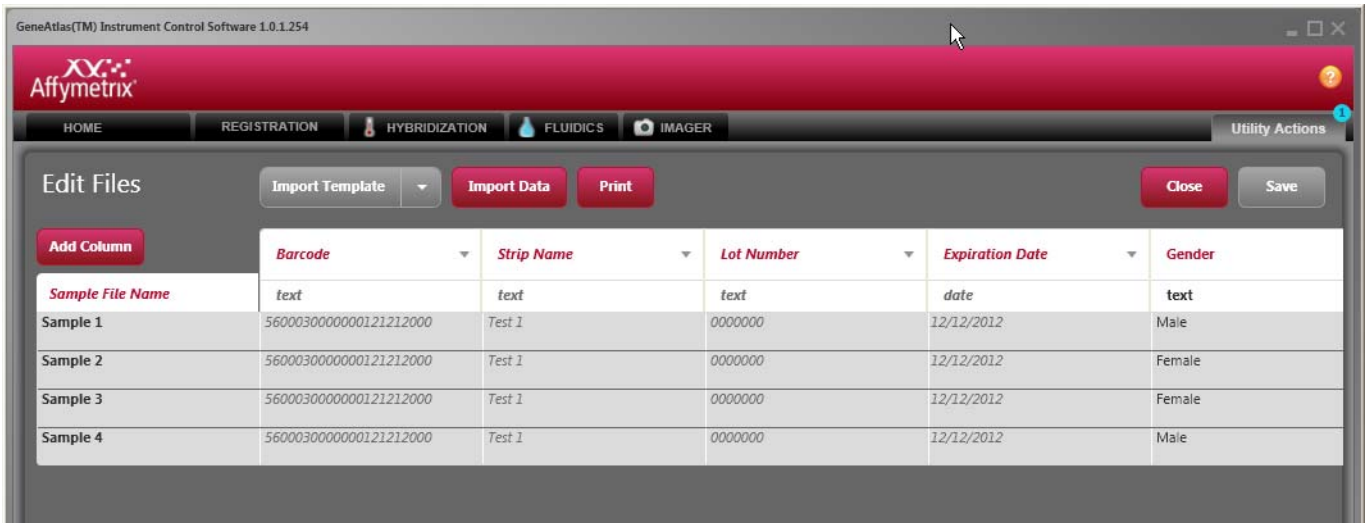
Figure 3.23 Select one or more sample files to open



2. In the Open File dialog box that appears, select the sample files that you want to modify, and click **Open**.
   The Edit Files window appears (Figure 3.24).

NOTE: If you plan to import a template, select all of the sample files of an Array Strip.

Figure 3.24 Edit Files window



3. If you want to:

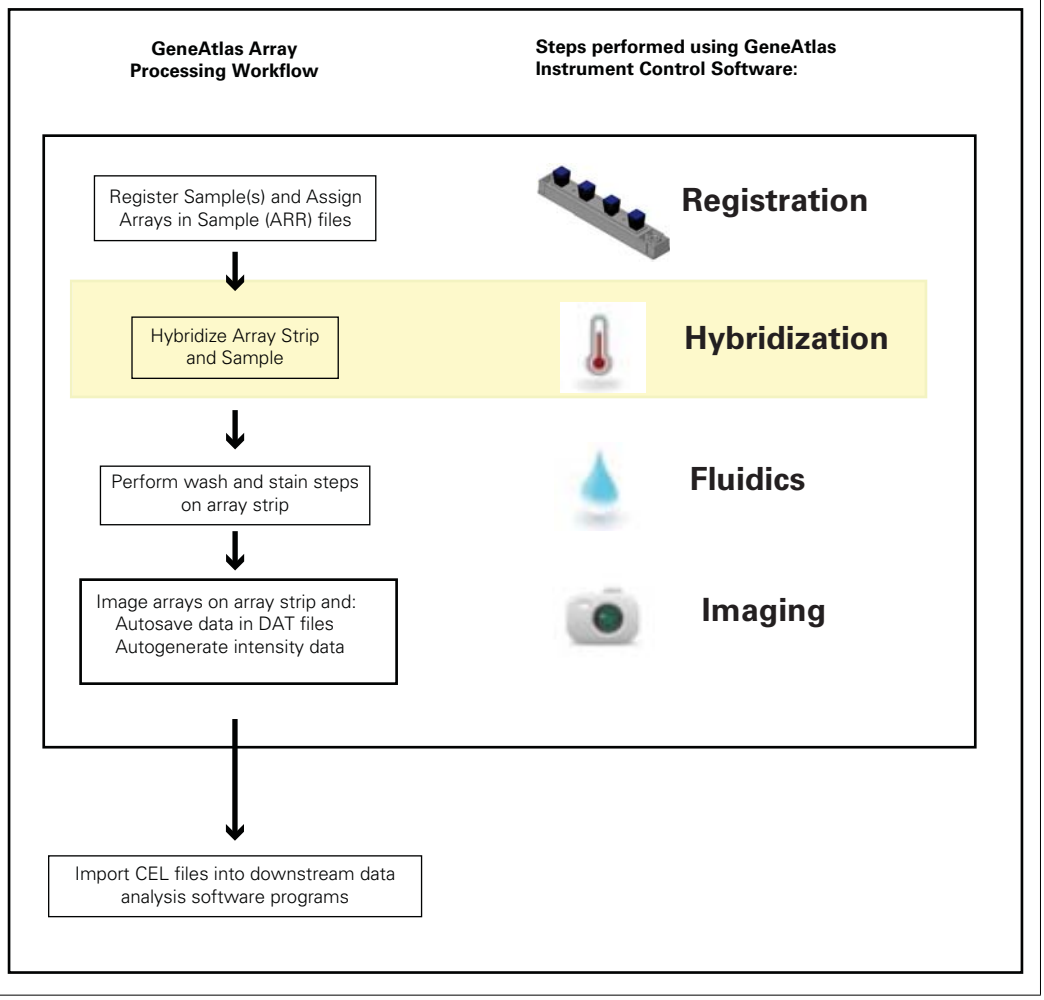
- Add a sample attribute to the data grid - See Step 9 on page 25
- Import a template - See Step 2 to Step 3 on page 28
- Import data - See Step 4 to Step on page 30
- Edit an attribute value - Select the item in the data grid and enter a new value

NOTE: In the Edit Files window, items that are shaded in gray cannot be modified.

Hybridization

Hybridization is the second step in the array processing workflow (Figure 4.1), after registering the sample and array. It is done using the Hybridization Station.

Figure 4.1 Array Processing Workflow



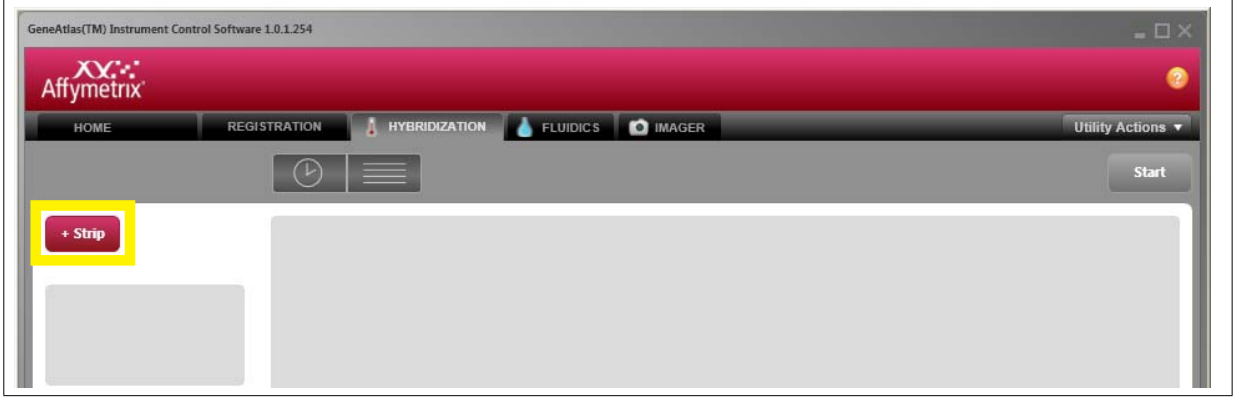
The GeneAtlas Hybridization tab allows you to track the array strips that are in hybridization. A timer notifies you when hybridization is finished and tracks the time since completion before the array strip was removed.

NOTE: The computer workstation does not control the hybridization station. The hybridization station *does not* shut down when hybridization is complete.

To perform hybridization:

1. Prepare samples for hybridization (see the Assay manual for details).
2. Register array strips and proceed to the Hybridization tab.
3. Click the **Hybridization** tab.

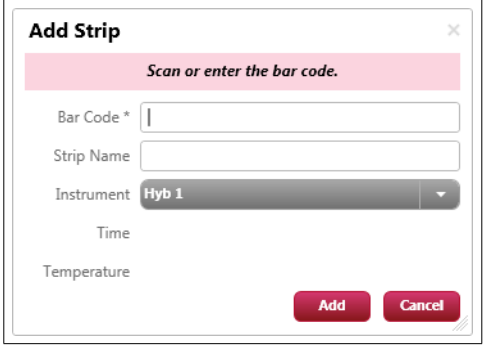
Figure 4.2 Hybridization window



4. Click the **+ Strip** button: 

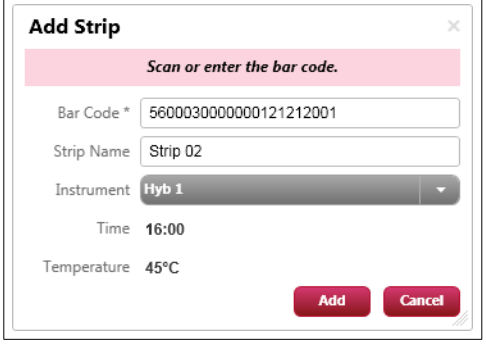
.
The Add Strip Window appears (Figure 4.3).

Figure 4.3 Add Strip for Hybridization dialog box



5. Scan or enter the **Bar Code** (required) of an Array Strip you have registered.
The **Strip Name** field is automatically populated.
6. From the **Instrument** drop-down box, select the correct hybridization station if multiple stations are available.
The **Time** and **Temperature** settings are automatically populated from the installed library files.

Figure 4.4 Add Strip for Hybridization dialog box

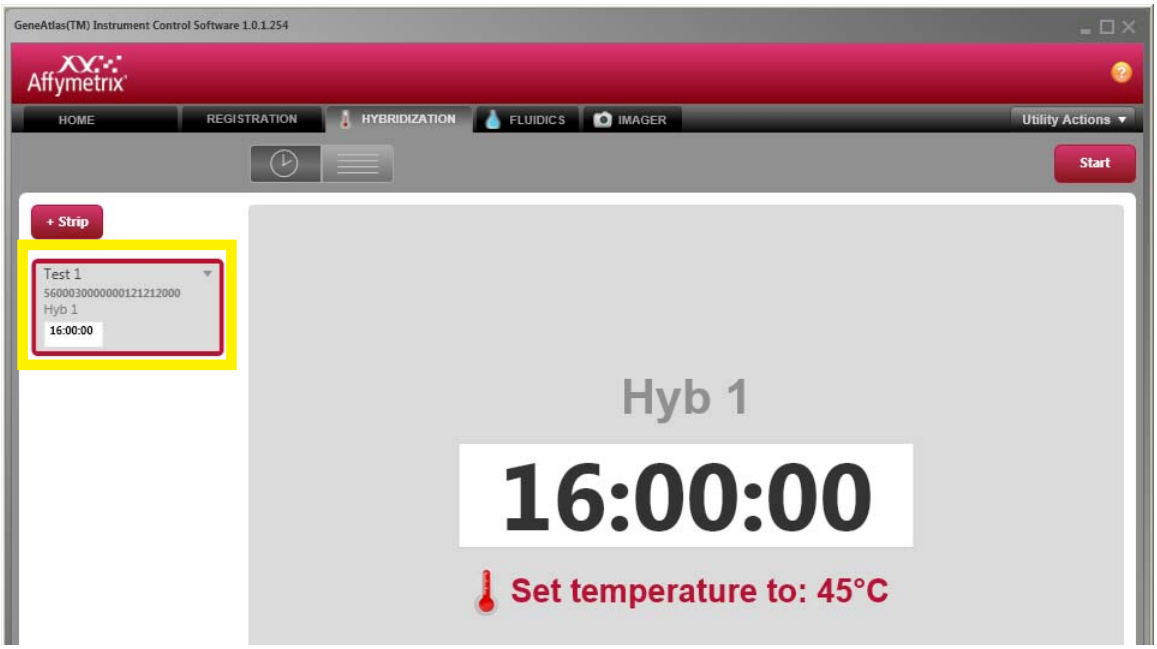


7. Hit the **Add** button in the Add Strip dialog box.

The strip is displayed on the left side of the screen.

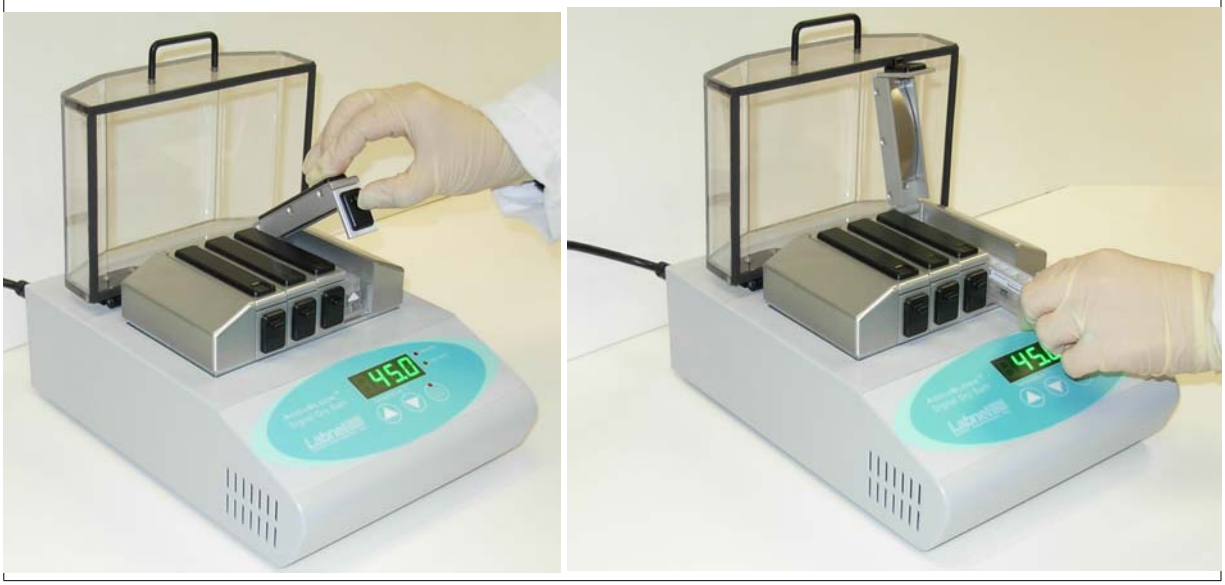
You can enter up to four arrays for hybridization.

Figure 4.5 Hybridization screen, strip entered



8. Set the Hybridization Station to the specified temperature. Before proceeding be sure that the Hybridization Oven has reached the correct temperature.
9. Open the hybridization station by pushing the front of the strip clamp towards the rear of the station and applying downward pressure on the top (Figure 4.6).
10. Place the hybridization tray with the array strip into a clamp inside the Hybridization Station. Refer to the Assay Manual for details.

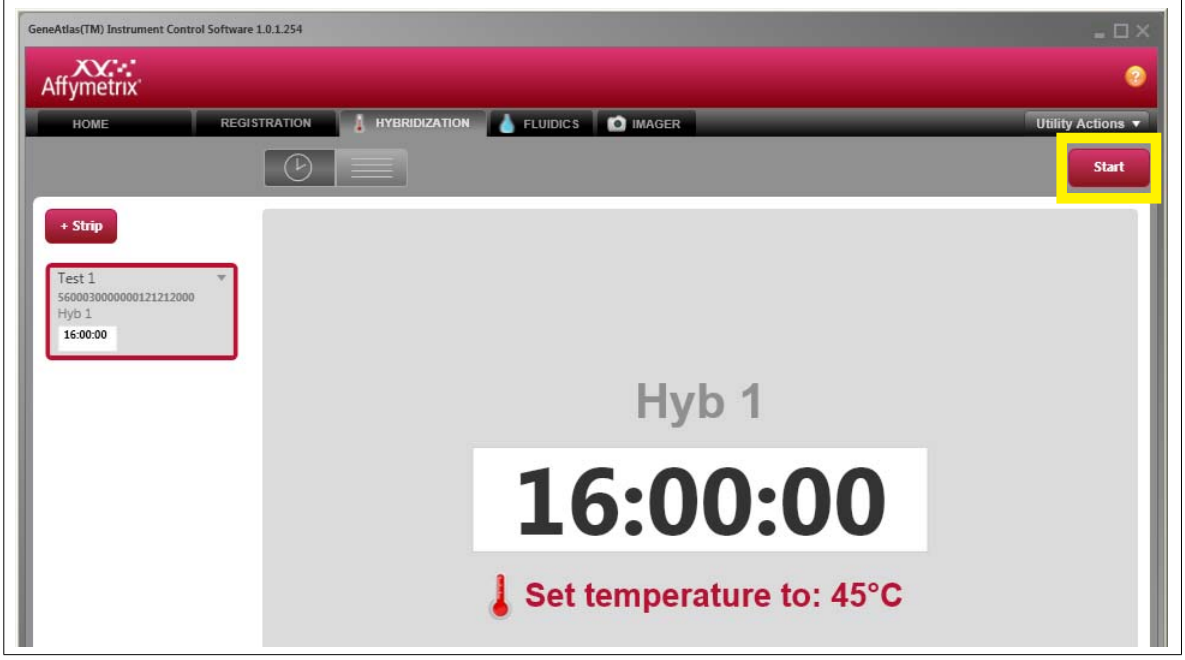
Figure 4.6 Opening hybridization unit cover and inserting array strip and Hybridization tray



11. Close the cover over the Array Strip

12. Click the **Start** button on the Hyb tab (Figure 4.7).

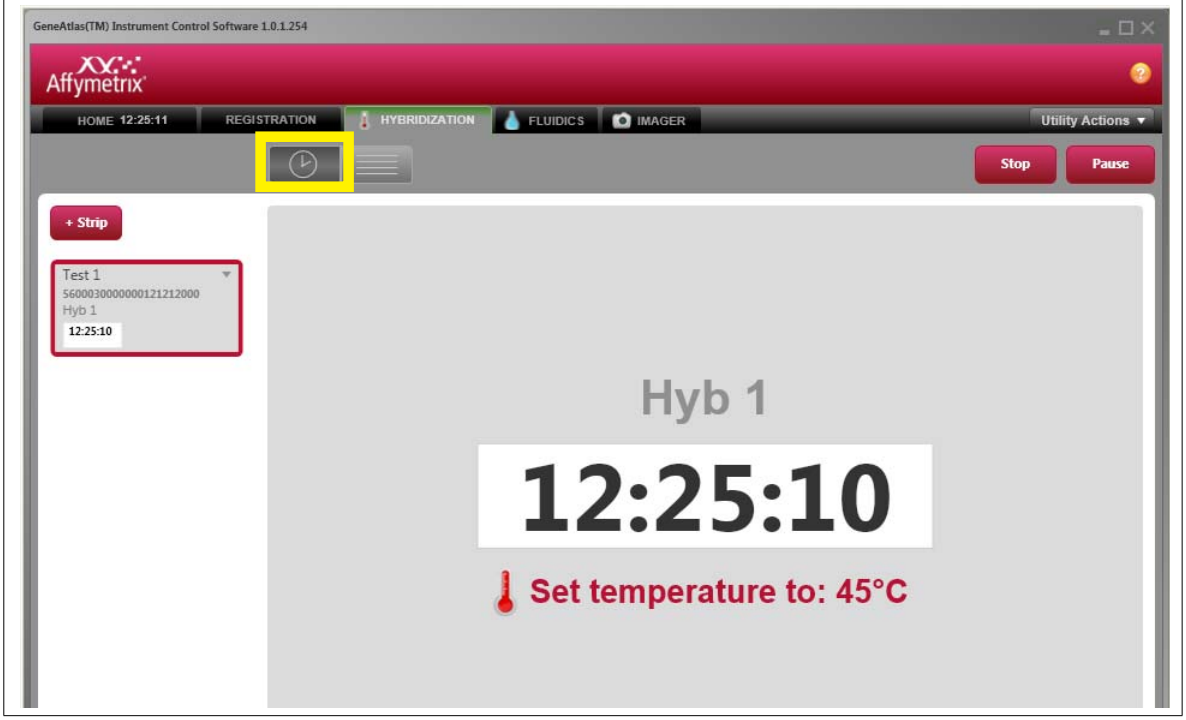
Figure 4.7 Hybridization screen, strip entered



NOTE: Please label the hybridization station cover to identify the location of a specific array strip. The software does not track the location of a specific array strip, and premature removal of an array strip will affect results.

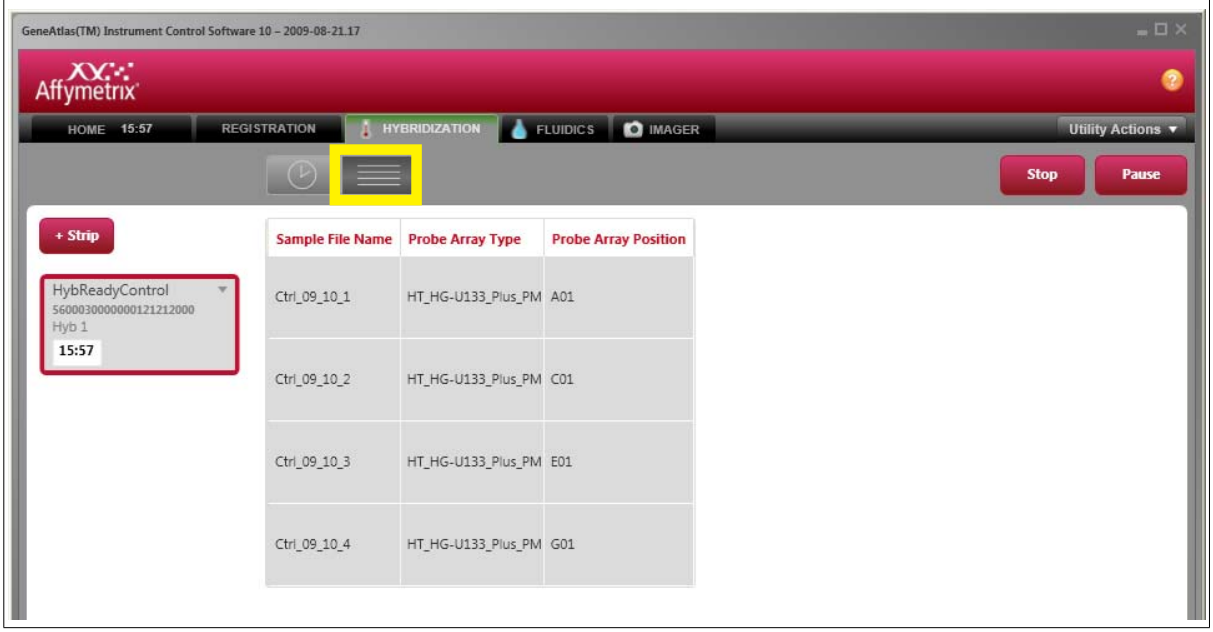
The software displays the hybridization time countdown. This time is displayed with a white background (Figure 4.8). When the countdown has completed the display turns yellow and the time begins to count up.

Figure 4.8 Hybridization Countdown



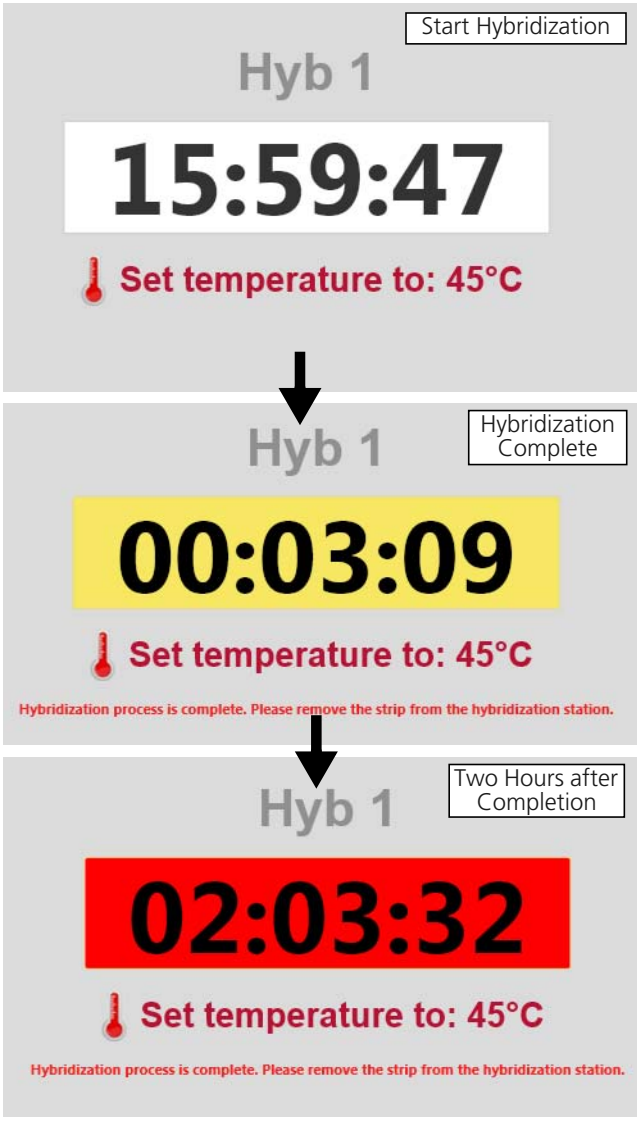
You can also click the **List** button to display a list of the samples being processed on the selected array strip. (Figure 4.9).

Figure 4.9 Hybridization tab, List view



When the timer begins counting the area around the timer is white. When hybridization is complete the area around the timer changes to yellow. Two hours after hybridization is complete the time changes from yellow to red (Figure 4.10).

Figure 4.10 Color changes with time in Hybridization tab

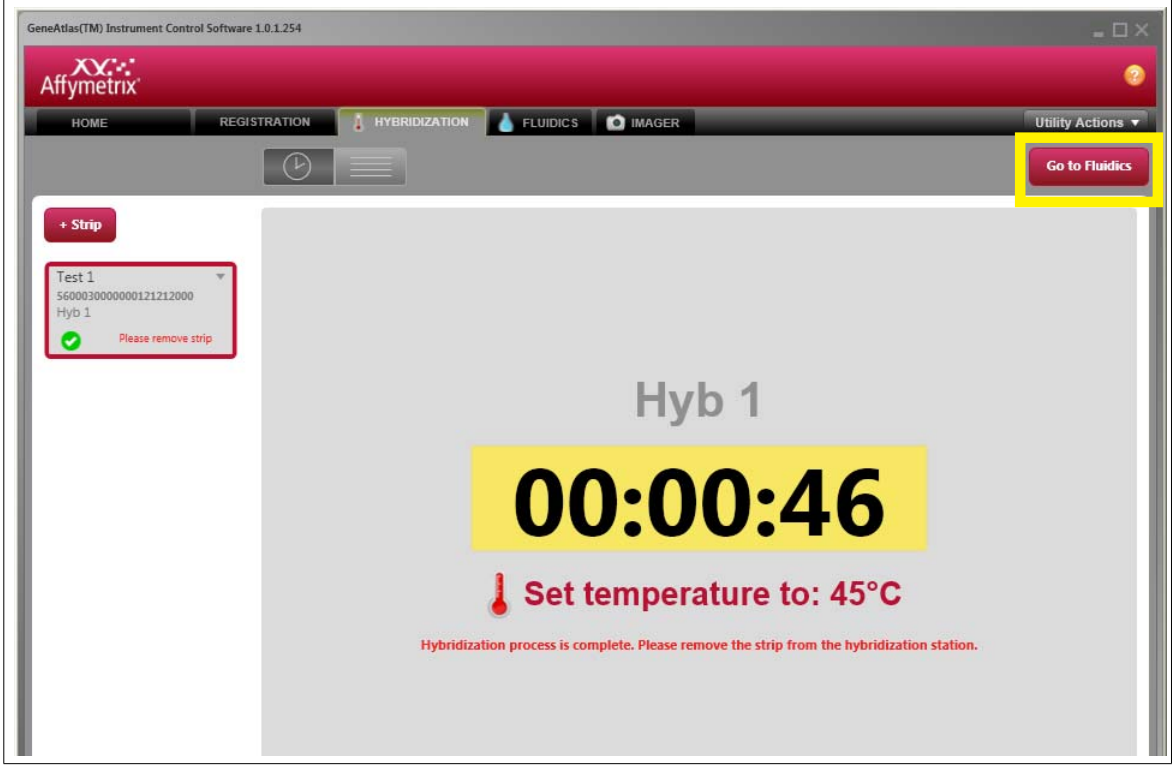


13. When hybridization has completed, click the **Stop** button in the upper right corner. A confirmation message box appears (Figure 4.11).

Figure 4.11 Confirmation Message

14. Click **Yes** to complete hybridization.
The **Go to Fluidics** button is active (Figure 4.12).

Figure 4.12 Go to Fluidics button activated.



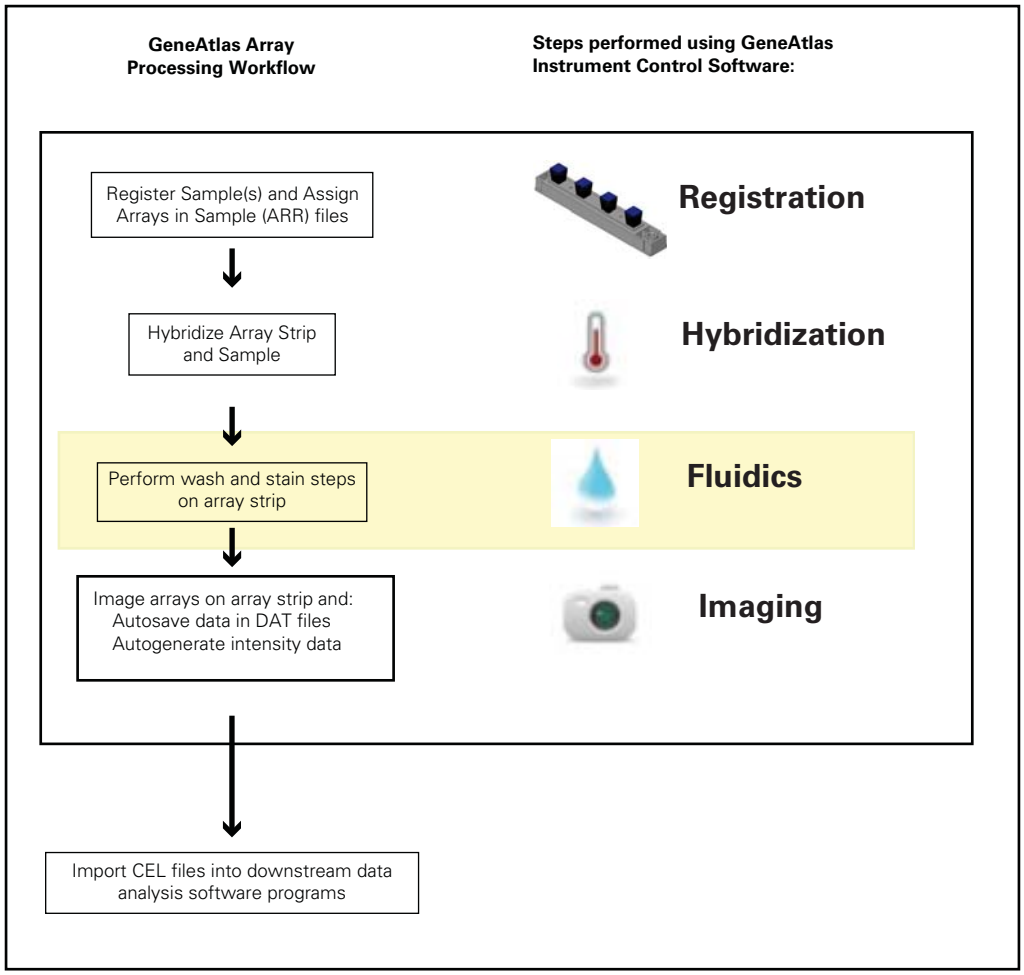
15. Click **Go To Fluidics**, or click the Fluidics tab to proceed to the next step.

IMPORTANT: **Going over the recommended hyb time may cause changes in data quality. Affymetrix recommends processing the arrays through fluidics and imaging as soon as hybridization is finished.**

Fluidics (Wash and Stain)

Fluidics is performed using the GeneAtlas Fluidics Station and the GeneAtlas Instrument Control software (Figure 5.1).

Figure 5.1 Array Processing Workflow



It requires two sets of steps:

- *Preparing Reagents and Filling Trays* on page 45
- *Loading Fluidics Station and Setting Up Run* on page 49
- Make sure to follow instructions in Chapter 4, *Hybridization* on page 35 to finish the hybridization.

Figure 5.2 The GeneAtlas® Fluidics Station

Preparing Reagents and Filling Trays

You first need to prepare reagents and fill the wells in the various trays for the Fluidics processing. These steps are described in the following sections:

- *Types of trays*
- *Required Supplies and Instruments*
- *Preparation of Trays*

Types of trays

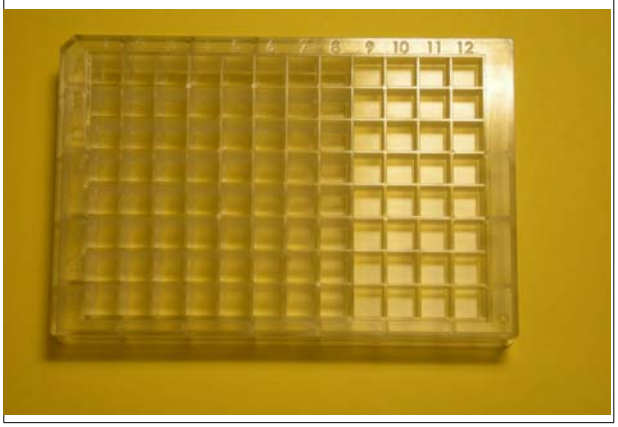
You need to prepare the following trays before beginning the Fluidics processing:

- GeneAtlas Wash B Tray (P/N 2022256)

Figure 5.3 Wash B Tray

- GeneAtlas Wash A/Stain Tray (P/N 202257)

Figure 5.4 Wash A/Stain Tray



- GeneAtlas Imaging Tray (P/N 5600004)

Figure 5.5 Imaging Tray with cover

Required Supplies and Instruments

Table 5.1 Reagents

Reagent	List No.	Spec	Product Factory	Storage
Wash and Stain Kit	900720	30 rxns	Affymetrix	4°…

Table 5.2 Instruments

Instruments	Type	Product Factory
Microcentrifuge	5415D	Multiple sources
Vortex-Mixer	TDX-1	Multiple sources
Micropipettes	P-2,P-20,P-200,P-1000	Multiple sources
Sample Tube Rack	0.2 mL, 0.5-1.5 mL	Multiple sources
GeneAtlas® Fluidics Station		Affymetrix
GeneAtlas® Imaging Station		Affymetrix

Table 5.3 Consumables

Consumable	Type	Product Factory
Disposable Tip	0.5-10 μL, 2-200 μL,100-1000 μL	Multiple sources
Disposable Tube	0.2 mL, 1.5 mL	Multiple sources
Imaging tray	/	Affymetrix
WashA/Stain tray	/	Affymetrix
WashB tray	/	Affymetrix

Preparation of Trays

NOTE: You can use the GeneAtlas® IVT/WT Fluidics Station Quick Reference Card (P/N 08-0310) or the GeneAtlas® miRNA Fluidics Station Quick Reference Card (P/N 703113) as a convenient reference when filling the trays.

To prepare trays for a fluidics run:

1. Prepare wash and stain reagents twenty minutes before running the fluidics module.

   NOTE: The reagents need to be acclimatized to room temperature before using.

2. Take the following reagents from Stain Module (Box 1) and shake gently taking care to avoid bubble formation:
   - Stain Cocktail 1
   - Stain Cocktail 2
   - Array Holding Buffer.

   NOTE: You may use a repeating pipettor to speed up the process.

   NOTE: If you are careful, you can fill the trays without having bubbles form. If you get bubbles, you can use a pipet tip to pop them (this works if you have 1 bubble in the corner, for example).

   NOTE: For WT and IVT array products, use tray layout map shown in Figure 5.6 on page 48. For miRNA array products, use tray layout map shown in Figure 5.7 on page 48

3. Add 850 µL of Wash B to the corresponding positions on the Wash B Tray (Figure 5.6 or Figure 5.7).
4. Add 200 µL of Array Holding Buffer to the corresponding positions in the Imaging tray (Figure 5.6 or Figure 5.7).
5. Add 850 µL of Wash A to the corresponding positions in the Wash A/Strain Tray (Figure 5.6 or Figure 5.7).
6. Add 350 µL of Stain Cocktail 1 to the corresponding positions in the Wash A/Stain Tray (Figure 5.6 or Figure 5.7).

   Stain Cocktail 1 is pinkish in color due to presence of fluorescent dye.
7. Add 350 µL of Stain Cocktail 2 to the corresponding positions in the Wash A/Stain Tray (Figure 5.6 or Figure 5.7).

Figure 5.6 IVT/WT Tray maps

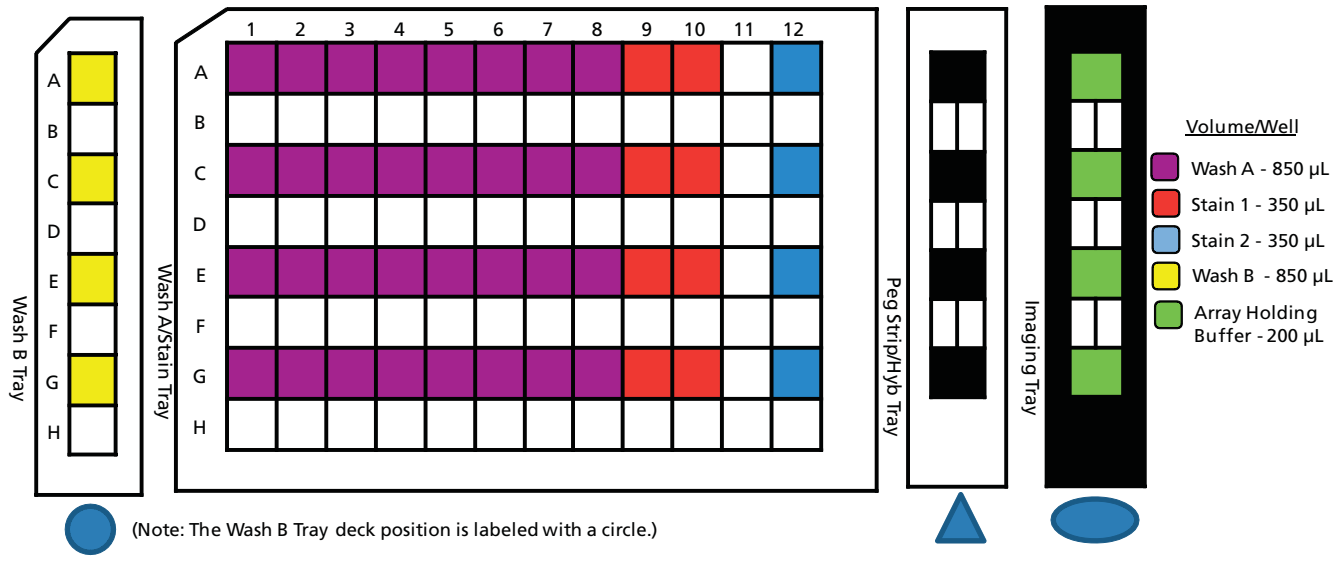
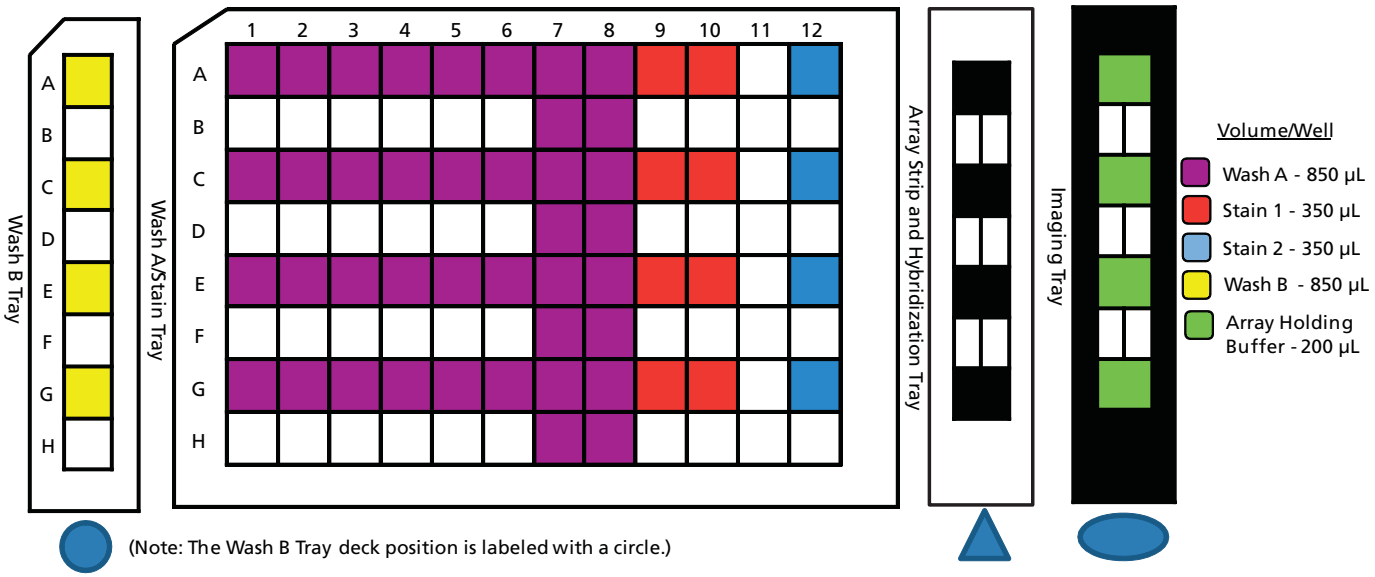


Figure 5.7 miRNA Tray maps



Loading Fluidics Station and Setting Up Run

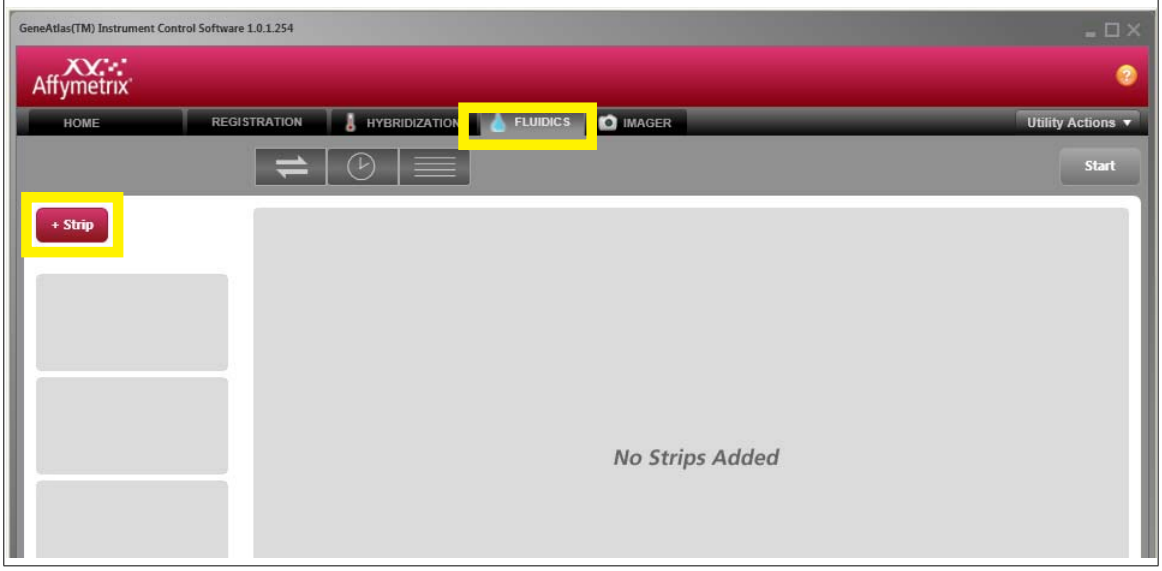
After preparing the consumable trays for the fluidics run, you need to:

- Display the Fluidics tab
- Add the strip
- Load the trays
- Start the run.

To run the Fluidics step in the workflow:

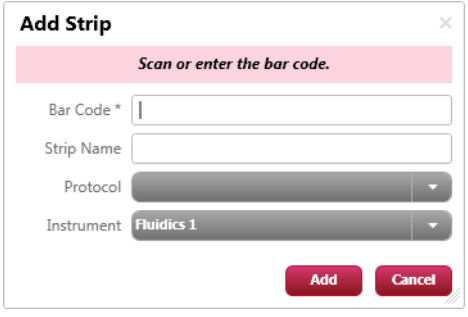
1. Make sure the Fluidics tab is displayed:
   It will be automatically displayed if you have come to this step directly from the Hybridization step; otherwise, click the Fluidics tab to display the controls.
2. In the Fluidics tab (Figure 5.8), click the **+ Strip** button: + Strip.

Figure 5.8 Fluidics tab



The Add Strip Window appears (Figure 5.9).

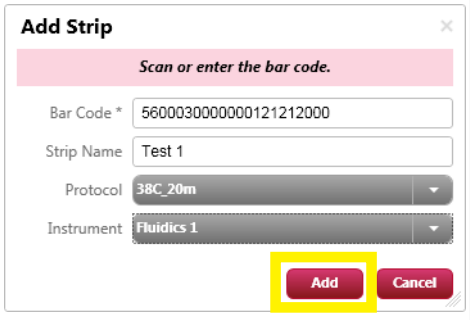
Figure 5.9 Add Strip for Fluidics



3. Scan or manually enter the **Bar Code** (required) of an Array Strip you have registered.
   The **Strip Name** field is automatically populated.
   The appropriate protocol will be selected by the application. If you wish to run a custom protocol, you may select it from the dropdown list.

4. From the **Instrument** drop-down box, select the proper instrument if more than one is installed.

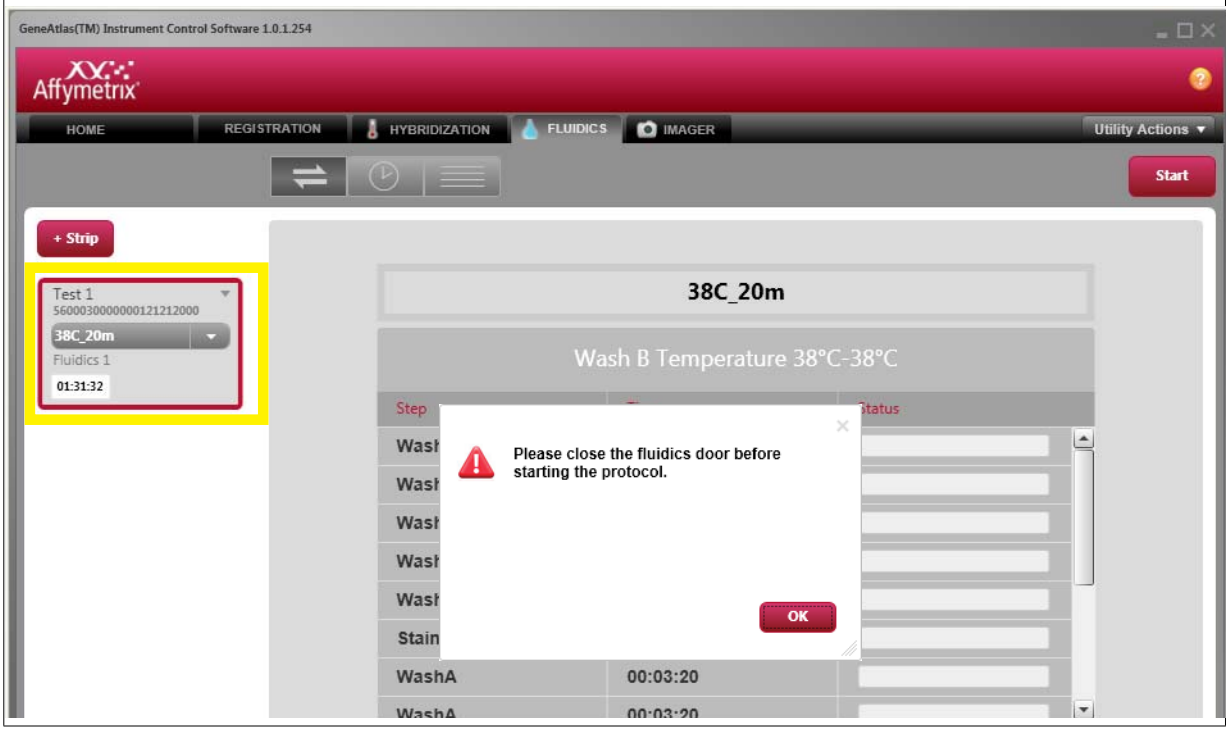
Figure 5.10 Protocol and Instrument selected



5. Click the **Add** button in the Add Strip dialog box (Figure 5.10).

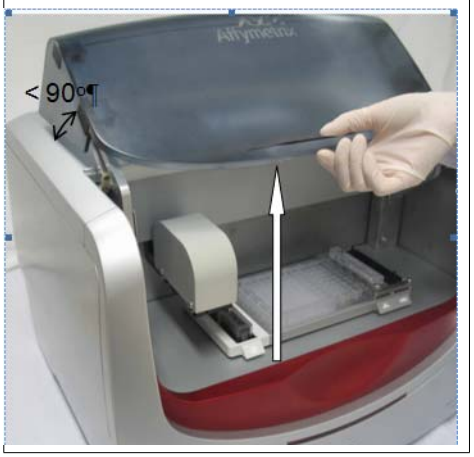
   The array strip information is displayed on the left side of the Fluidics tab and a notice to close the fluidics door before starting the procedure is displayed (Figure 5.11).

Figure 5.11 Strip information



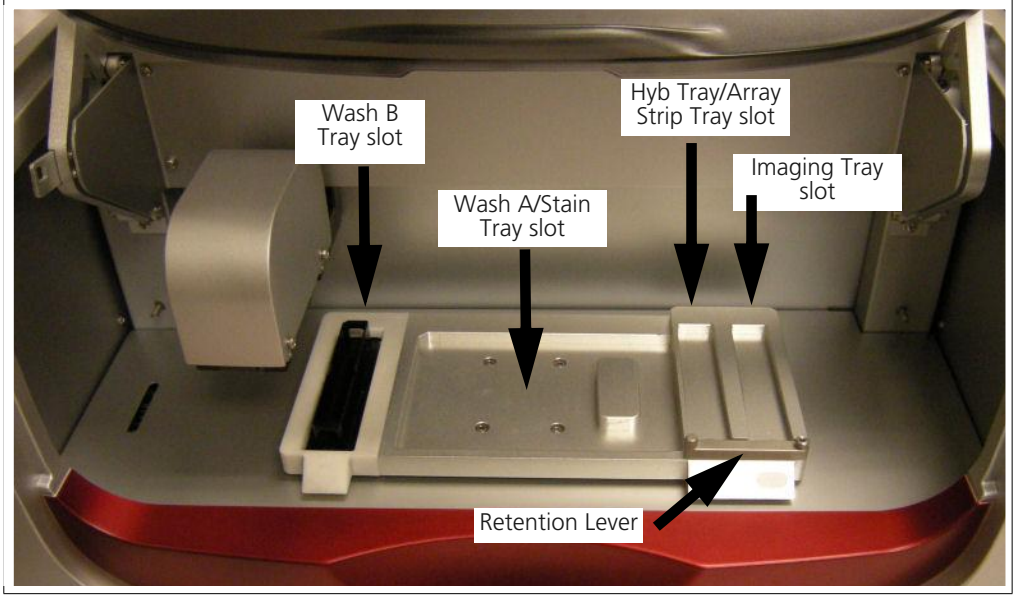
6. Gently open the cover of the Fluidics station (Figure 5.12).

Figure 5.12 Opening the Fluidics Station door



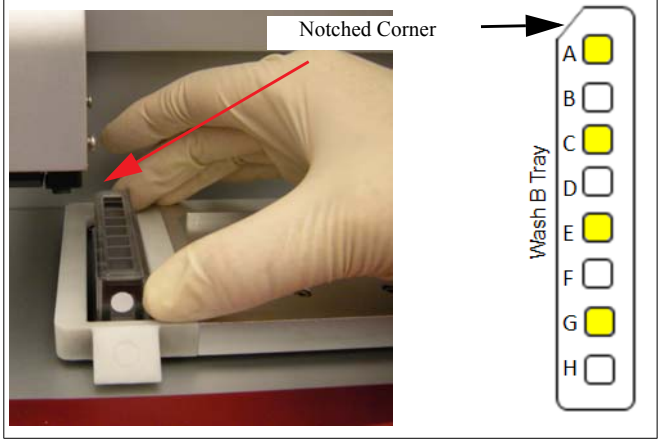
The trays need to be placed on the Fluidics Station deck (Figure 5.13).

Figure 5.13 Fluidics Station Tray Deck



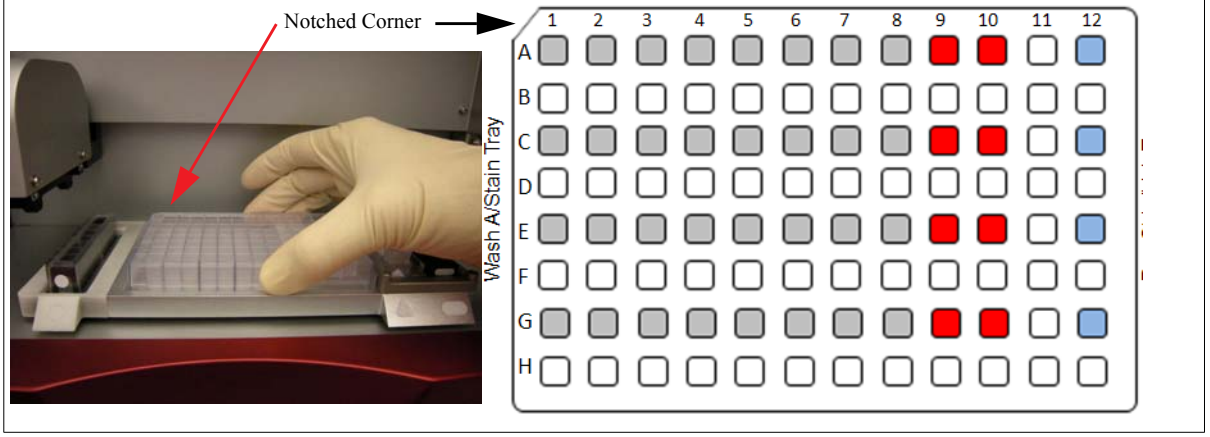
7. Load the Wash B tray (Figure 5.14). Make sure it is seated properly.

Figure 5.14 Load Wash B tray



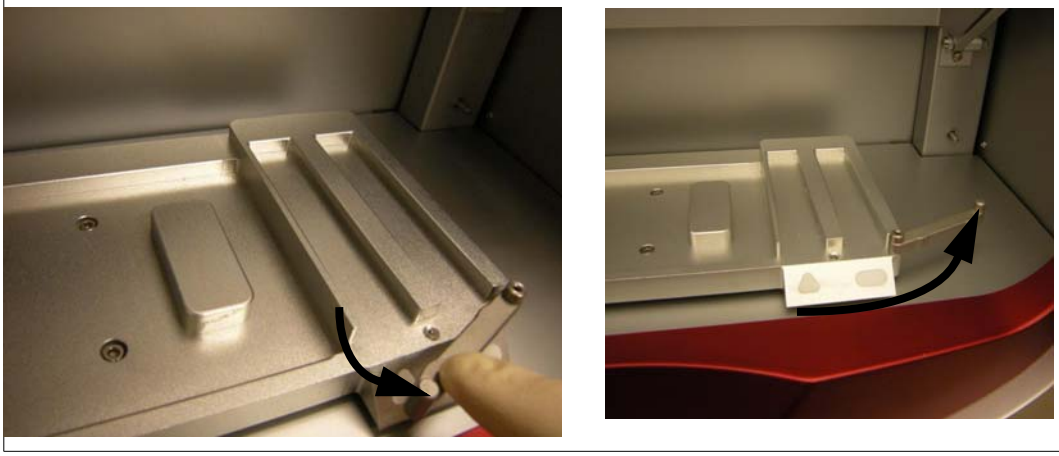
8. Load the Wash A tray (Figure 5.15). Gently push the tray to the notched (upper left) corner after seating.

Figure 5.15 Load Wash A tray



9. Open the retaining lever in front of the Hybridization Tray and Imaging Tray locations on the Fluidics Station Tray Deck by rotating the left-hand side of the lever in the counter-clockwise direction (Figure 5.16).

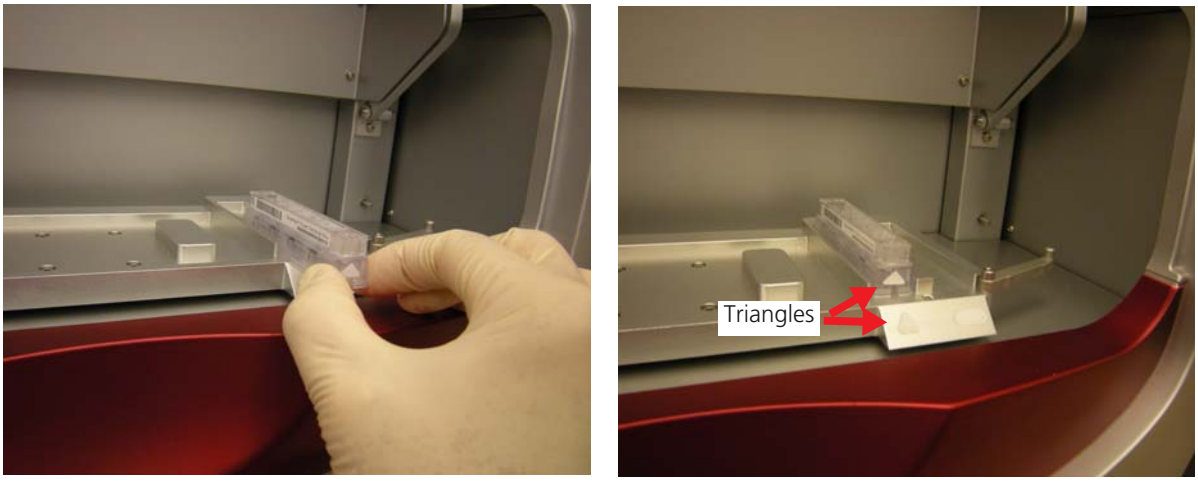
Figure 5.16 Opening the retaining lever



IMPORTANT: The positioning label shapes on the Tray Deck and on the plastic trays are slightly different.

10. Load the mated Hyb tray and array strip on the deck of the Fluidics Station in the Position labeled with a Triangle (Figure 5.17). The label with the barcode should be facing left and the Triangle on the Hybridization Tray should be facing out (toward you).

Figure 5.17 Loading Hyb tray and array strip]



The Imaging Tray (Figure 5.18) has been filled with array holding buffer.

Figure 5.18 Imaging Tray and Protective cover

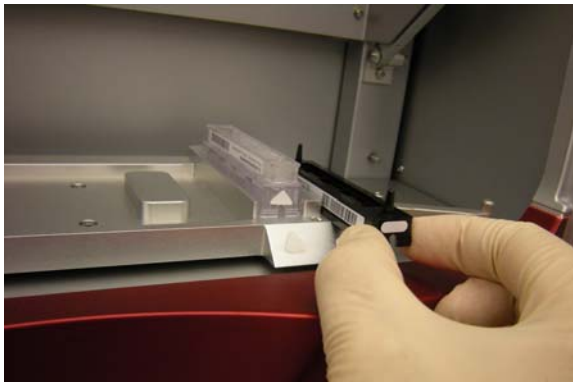
Imaging Tray

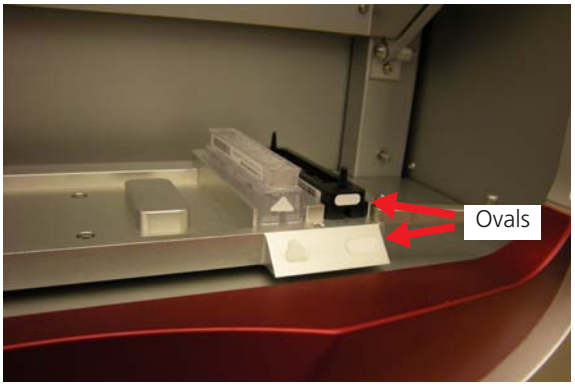
Protective Cover (Discard)

NOTE: Imaging Trays should always be handled with care. Do not touch the glass bottom of the tray.

11. Slide the Imaging Tray on the deck of the Fluidics Station in the Position labeled with a rectangle (Figure 5.19). The label with the barcode should be facing left and the oval on the Imaging Tray should be facing out (toward you).

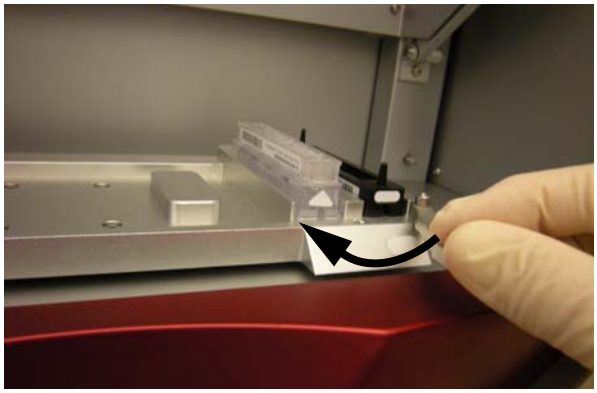
Figure 5.19 Loading Imaging Tray





12. Close the retaining lever in front of the Hybridization Tray and Imaging Tray locations by rotating the lever in the clockwise direction (Figure 5.20).

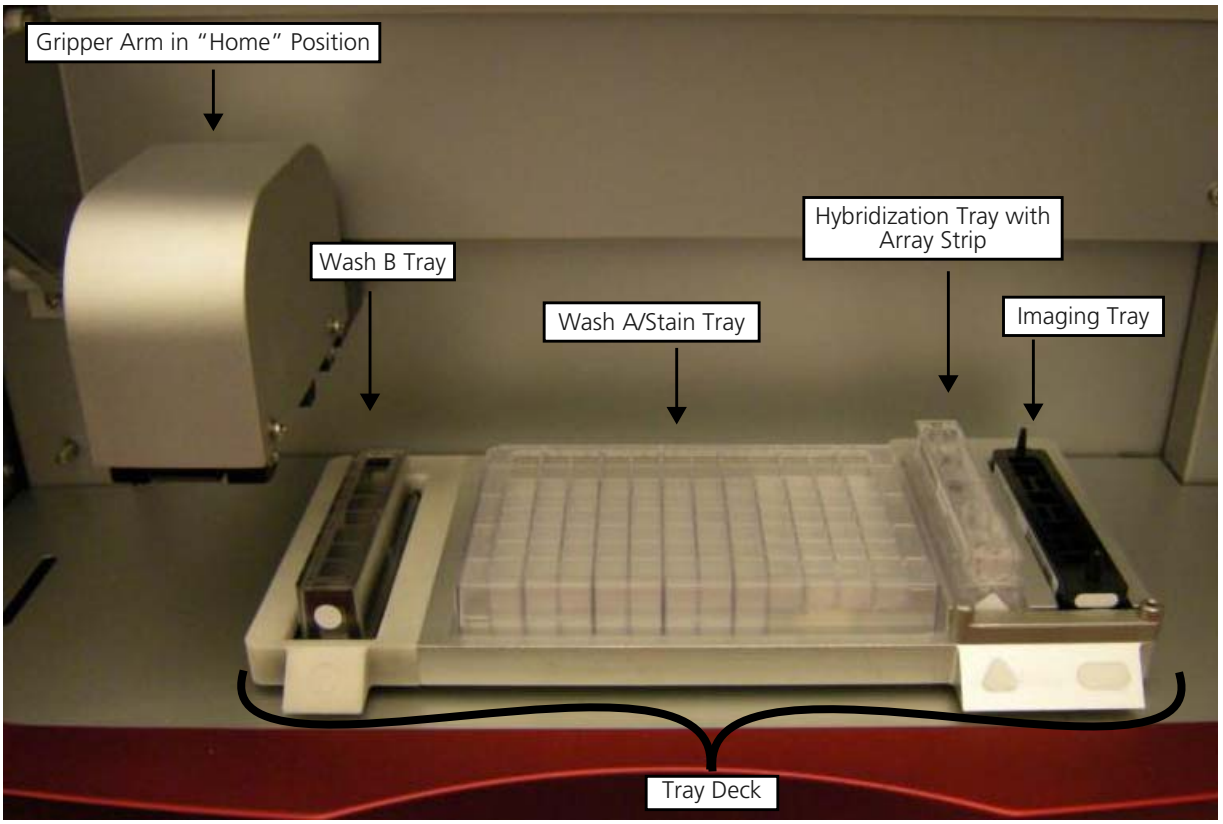
Figure 5.20 Closing lever (left) and lever closed (right)



WARNING: Improper alignment of the trays and array strip can cause instrument damage. Please ensure that all consumables are aligned properly. To ensure proper alignment, the trays should be pushed into the right-hand inner corner of their position on the deck of the Fluidics Station.

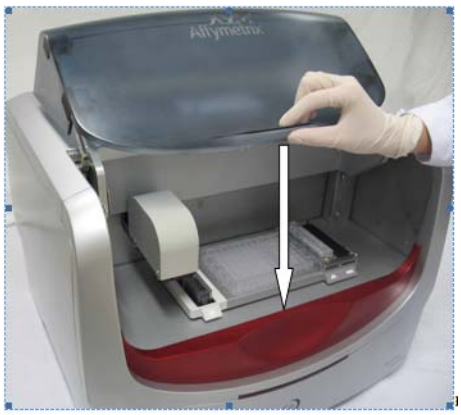
Be sure that labels and notched corners on both trays are facing to the left as shown in the image below (Figure 5.21).

Figure 5.21 Fluidics Station Tray Deck



13. Close the door of the Fluidics Station manually (Figure 5.22).

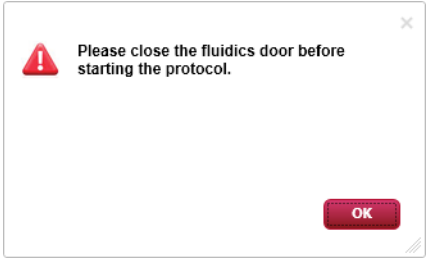
Figure 5.22 Closing the lid on the Fluidics station



After loading the Fluidics Station:

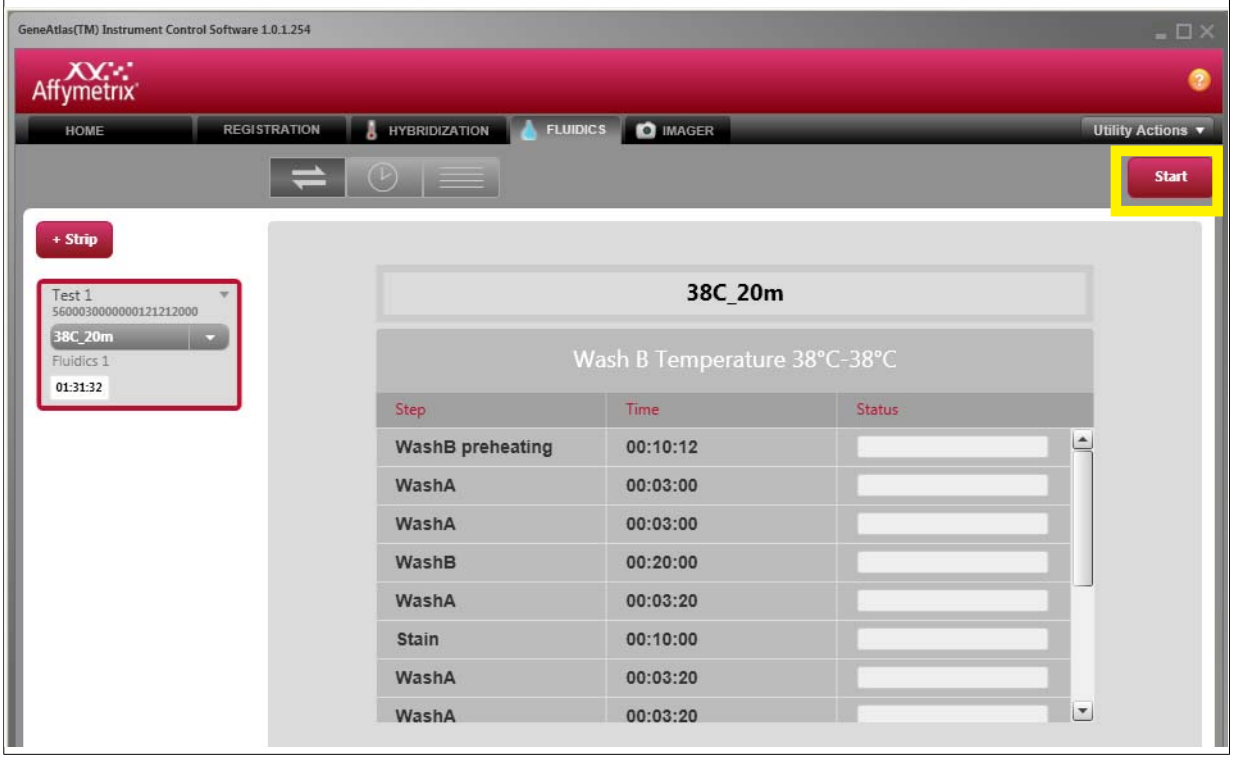
14. Click the OK button in the Close Fluidics Door notice (Figure 5.23).

Figure 5.23 Close Fluidics Door notice



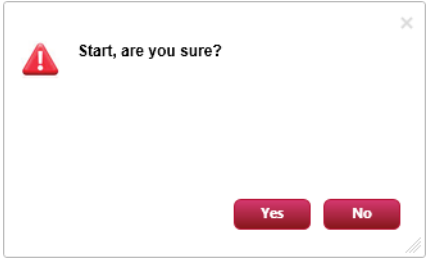
15. Click the **Start** button in the Fluidics tab (Figure 5.24).

Figure 5.24 Fluidics tab, Start button active



The Start notice appears (Figure 5.25).

Figure 5.25 Start Notice

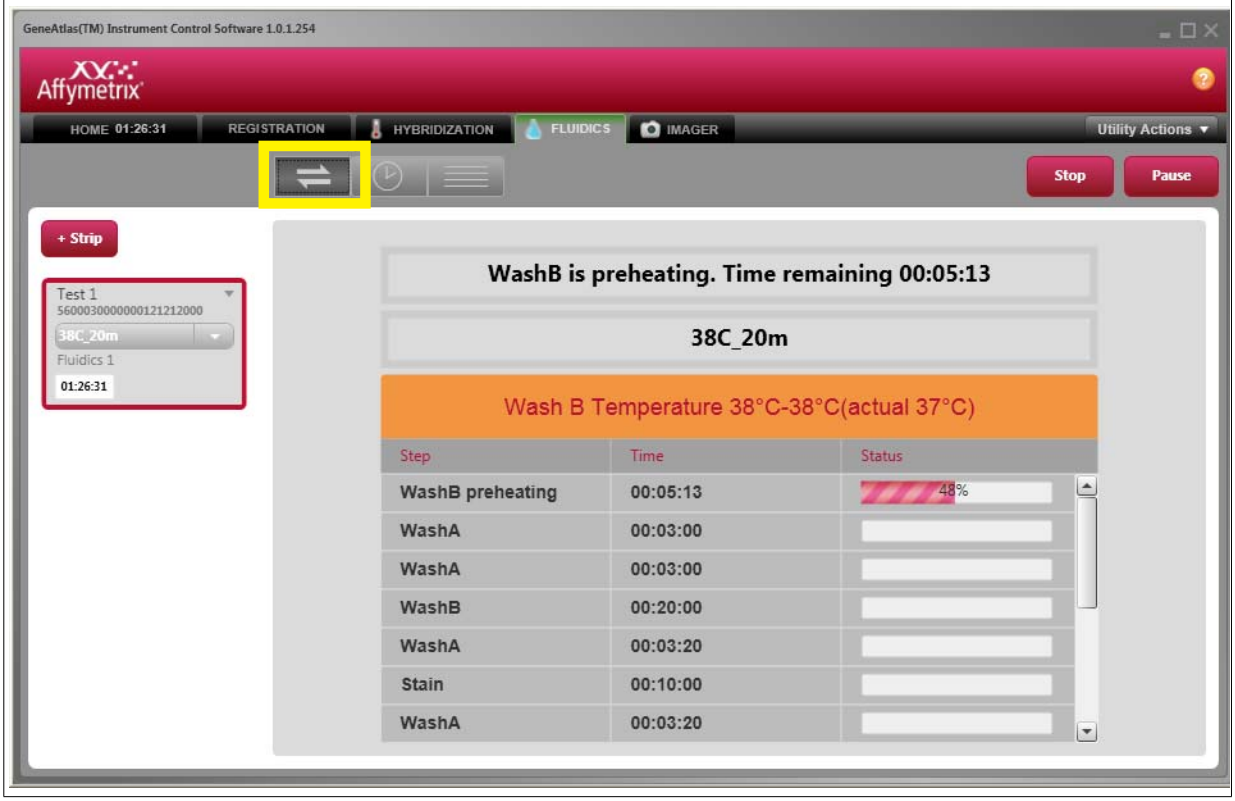


16. Click **Yes**.

After clicking the Yes button, the LED light on the front of the Fluidics Station flashes green to indicated that the unit has begun performing the protocol.

Protocol progress is shown in the Protocol view (Figure 5.26).

Figure 5.26 Fluidics tab, protocol progress displayed

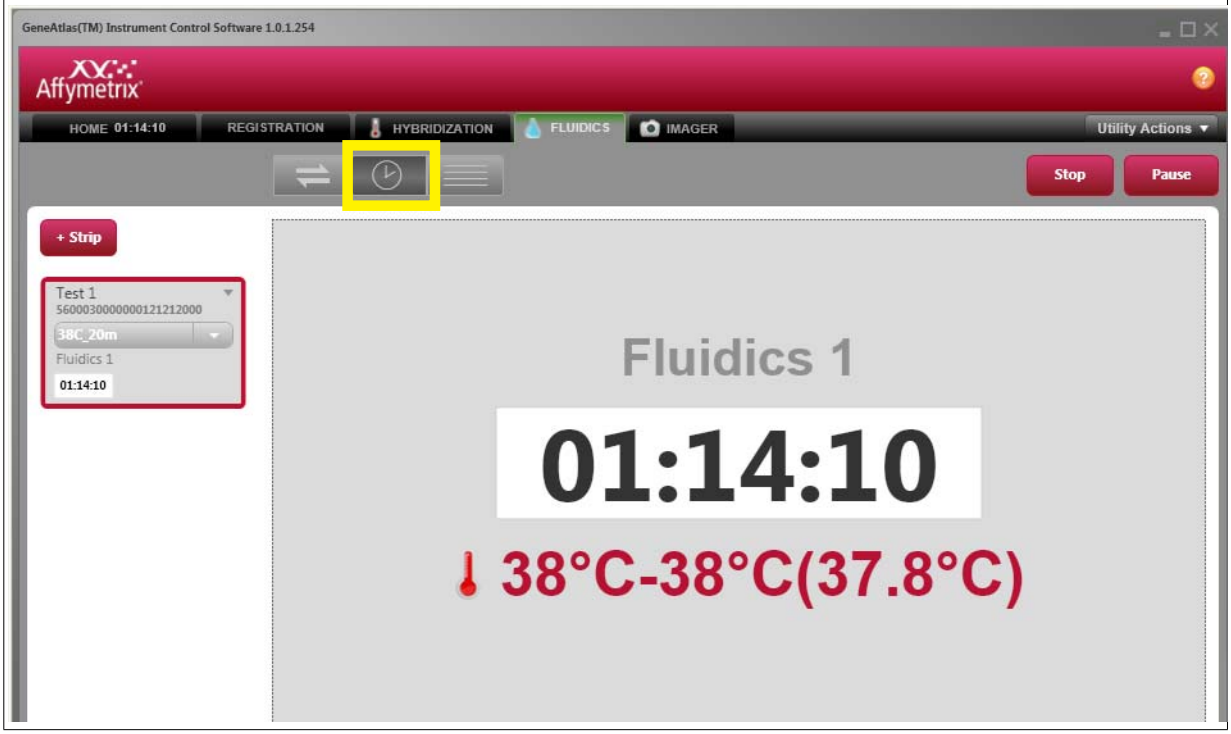


The Protocol View displays:

- Wash B temperature range and actual temperature
- Steps in protocol
- Time duration for each step
- Status of each step in the protocol

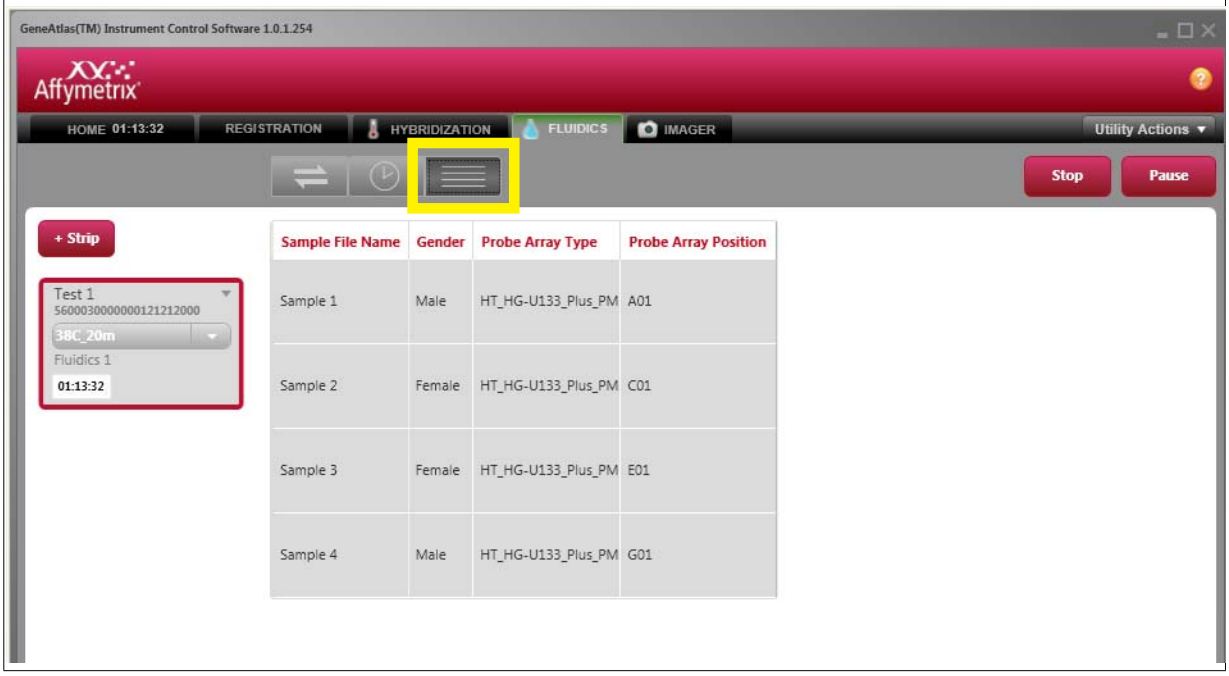
The Clock View (Figure 5.27) displays the time left for the overall fluidics step.

Figure 5.27 Fluidics tab, clock view



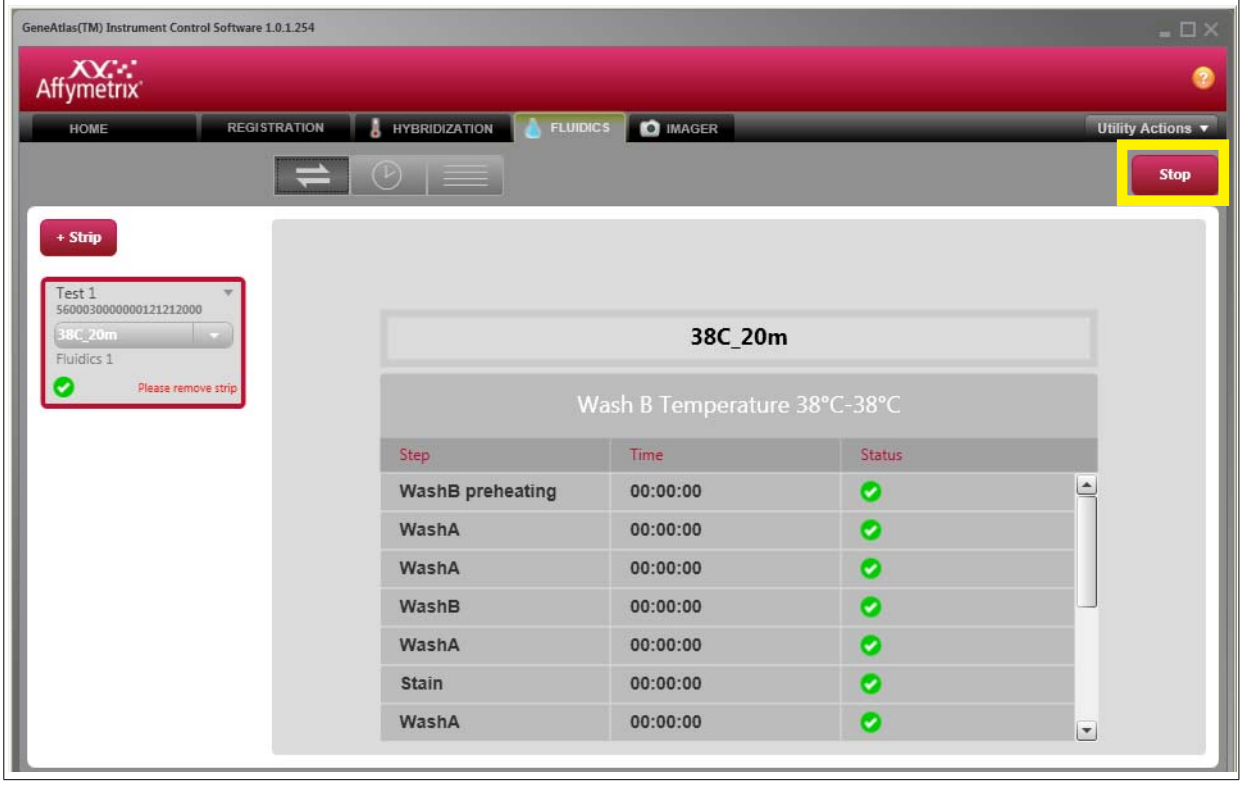
The List view (Figure 5.28) shows a list of the arrays on the strip being processed.

Figure 5.28 Fluidics tab, List view



When the Wash and Stain process is finished, you are notified on the user interface (Figure 5.29).

Figure 5.29 Notice of completion



17. Click **Stop**.

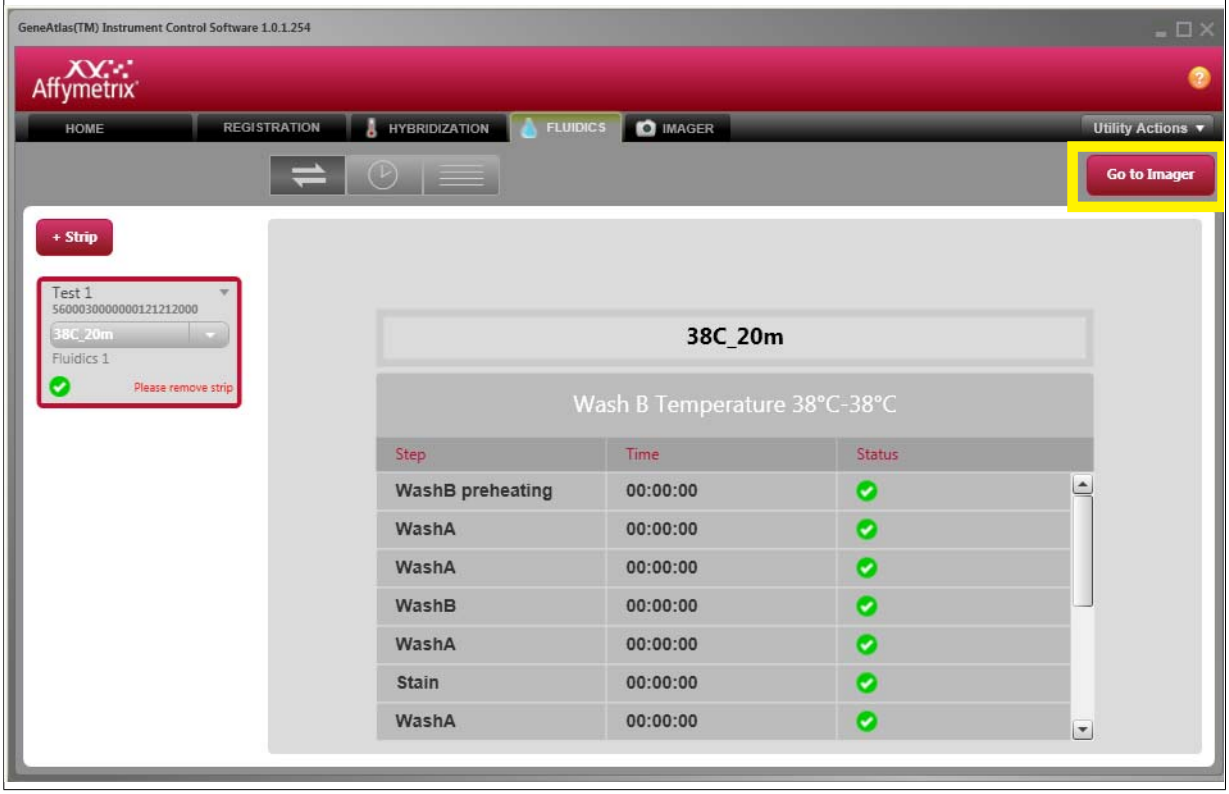
The Stop Notice opens (Figure 5.30).

Figure 5.30 Stop Notice

18. Click **Yes** to stop Fluidics run.

The Go to Imager button appears (Figure 5.31).

Figure 5.31 Fluidics tab with Go to Image button



19. Click **Go To Imager**.

    This automatically removes the Strip from the Fluidics tab. If you do not click the Go to Imager button, you will need to remove the strip from the tab manually.

20. Open the Fluidics Station.

21. Unlatch the Hyb Tray and Imaging Tray/Array Strip.

22. Remove the trays, disposing of the wash and hyb trays properly and placing the Imaging Tray and Array Strip in the Imaging Station.

IMPORTANT: Affymetrix recommends imaging the arrays as soon as the fluidics operation is finished.

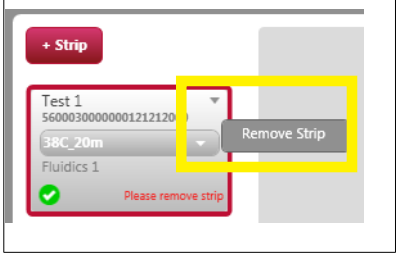
Removing Strip Manually from the Fluidics Tab

This step is necessary if you do not click the **Go To Imager** button when you finish the Fluidics processing.

To manually remove an array strip from the Fluidics tab:

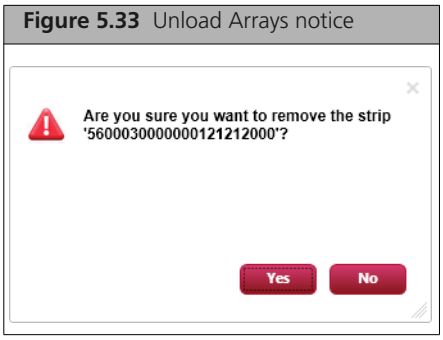
1. Click on the downward facing arrow in the Strip Information box on the left of the screen and select **Remove Strip** from the menu.

Figure 5.32 Remove Strip menu



The Remove Strip Notice appears (Figure 5.33).

Figure 5.33 Unload Arrays notice

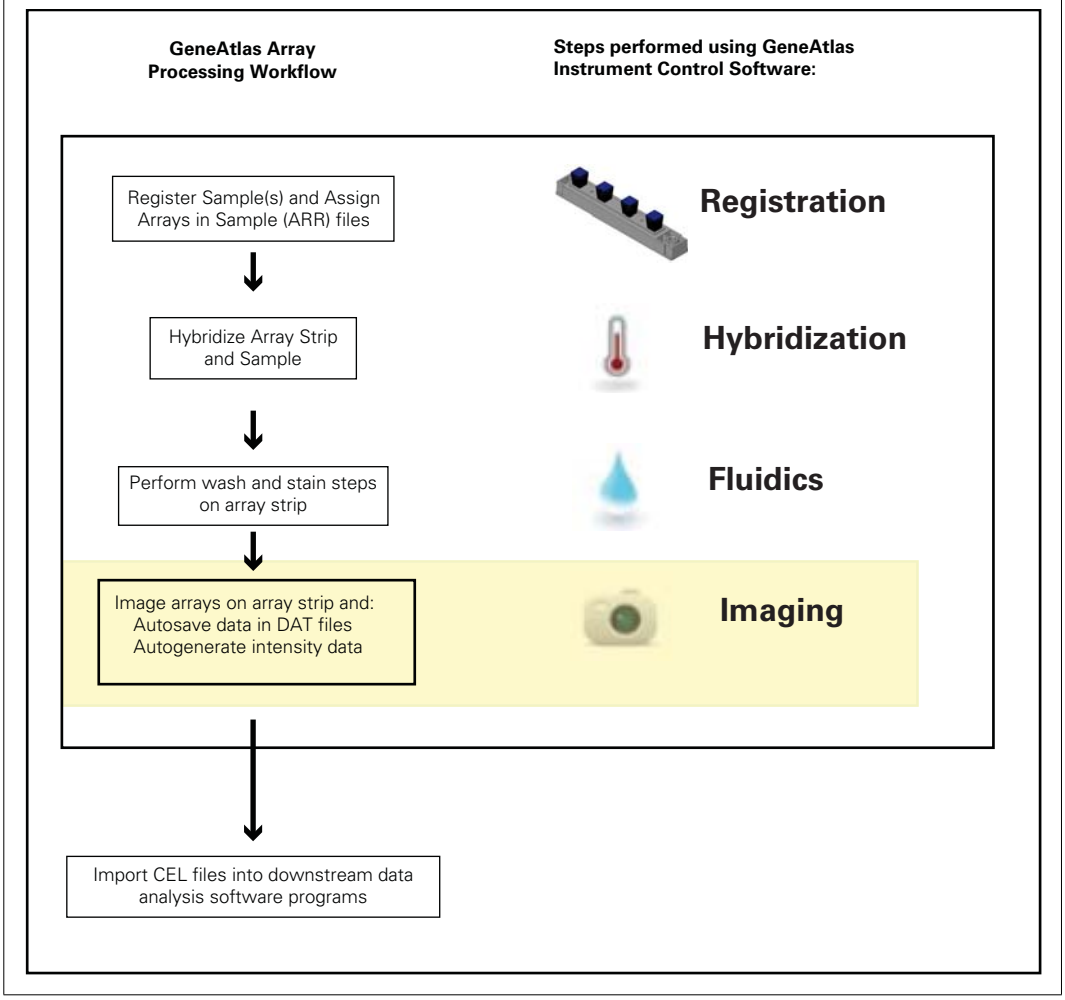


2. Click **Yes**.
   The array strip is removed from the system.

Imaging

Imaging the arrays is the fourth step in the array processing workflow (Figure 6.1).

Figure 6.1 Array Processing Workflow



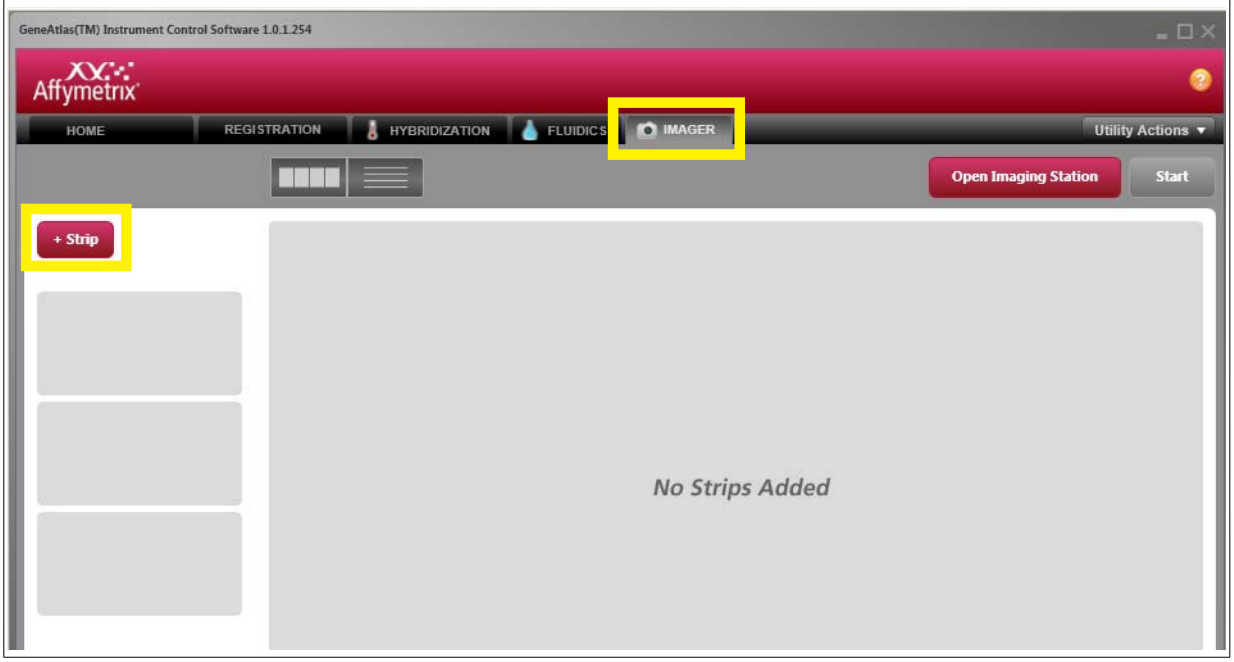
The GeneAtlas Imaging Station (Figure 6.2) is used to image the array strip.

Figure 6.2 The GeneAtlas® Imaging Station

To run the Imaging Step in the workflow:

1. Make sure the Imager tab is displayed (Figure 6.3):
   It will be automatically displayed if you have come to this step directly from the Fluidics step; otherwise, click the Imager tab to display the controls.

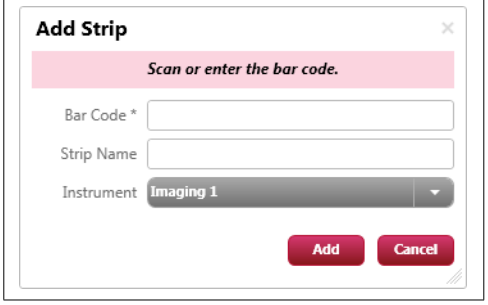
Figure 6.3 Imager window



2. Click the + **Strip** button: 

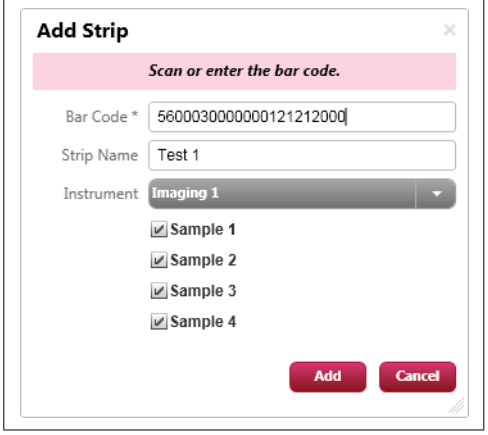
.
The Add Strip Window appears (Figure 6.4).

Figure 6.4 Add Strip dialog box for Imager



3. Scan or enter the **Bar Code** (required) of an Array Strip you have registered. The **Strip Name** field is automatically populated (Figure 6.5).

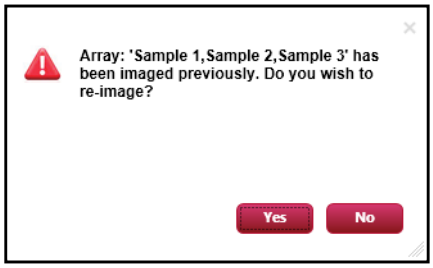
Figure 6.5 Add Strip dialog box for Imager



4. From the **Instrument** drop-down box, select the correct Imaging station if more than one is available.
5. Select the arrays that you wish to image (by default all are selected).
6. Click **Add**.

   The Array Strip information is displayed on the left side of the screen and the **Open Imaging Station** button is activated (Figure 6.6).

NOTE: If you are re-imaging a previously imaged array, the following notice appears:



Click *Yes* to re-image the array.

New data files will be created and named with the following format:

<Array Name>_<Re-Image Number>

For example, if the array *Sample* is re-imaged one time, the new data files will be named *Sample_1.*

The original data files will not be overwritten.

Figure 6.6 Imager tab, Open Imaging Station button activated

7. Click **Open Imaging Station** in the Imager tab.
   The Imaging Station door opens (Figure 6.7).

WARNING: Do not open the Imaging Station door manually. Doing so will damage the Imaging Station door.

Figure 6.7 Open Imaging Station door

8. Place the Imaging Tray and Array Strip into the Imaging Station (Figure 6.8).

Figure 6.8 Inserting Test Strip and Imaging tray

Be sure that the barcode on the Test Strip is facing to the left and that the oval on the imaging tray is facing out towards you.

9. Click the **Close Imaging Station** button on the Imager tab (Figure 6.9).

Figure 6.9 Imager tab, Close Imaging Station button displayed.

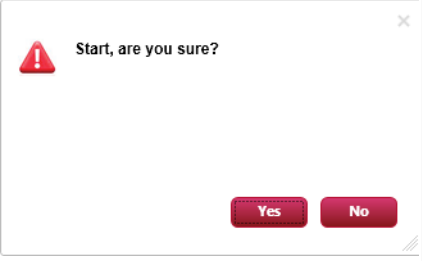
The Imaging Station door closes and the **Start** button is activated (Figure 6.10).

Figure 6.10 Imager tab, Start button activated.

10. Click the **Start** button in the Imager tab.

The Start Imaging Confirmation dialog box appears (Figure 6.11).

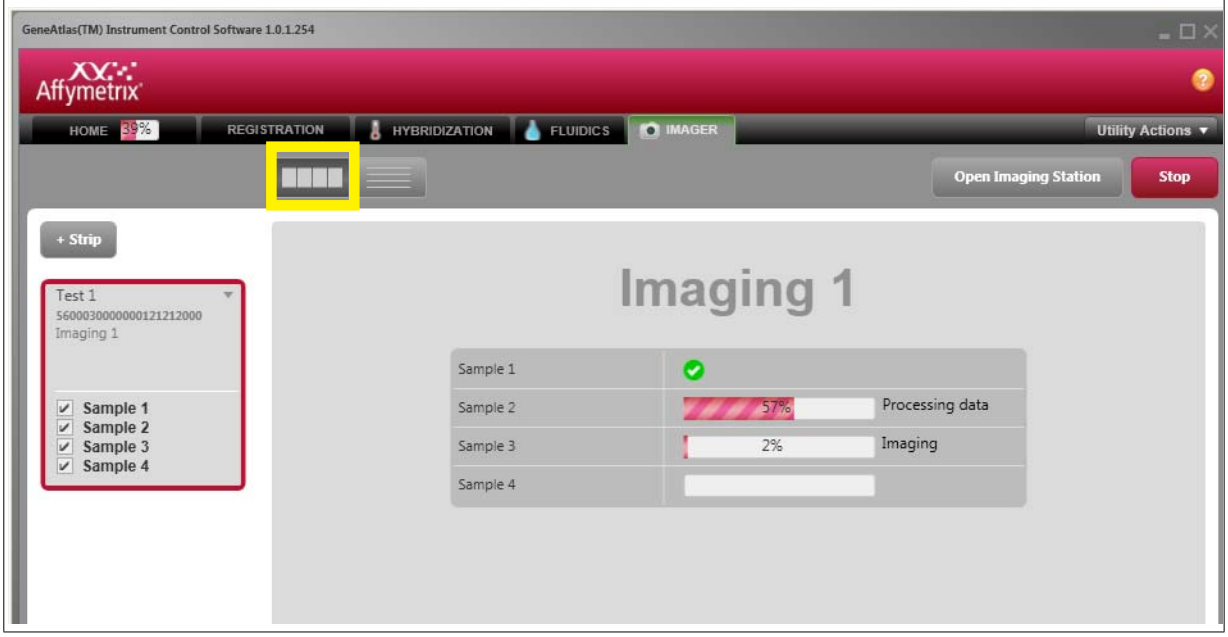
Figure 6.11 Start Imaging Confirmation dialog box



11. Click **Yes** to start imaging.

You can track the progress of imaging in the Progress view (Figure 6.12).

Figure 6.12 Imager tab, Progress view



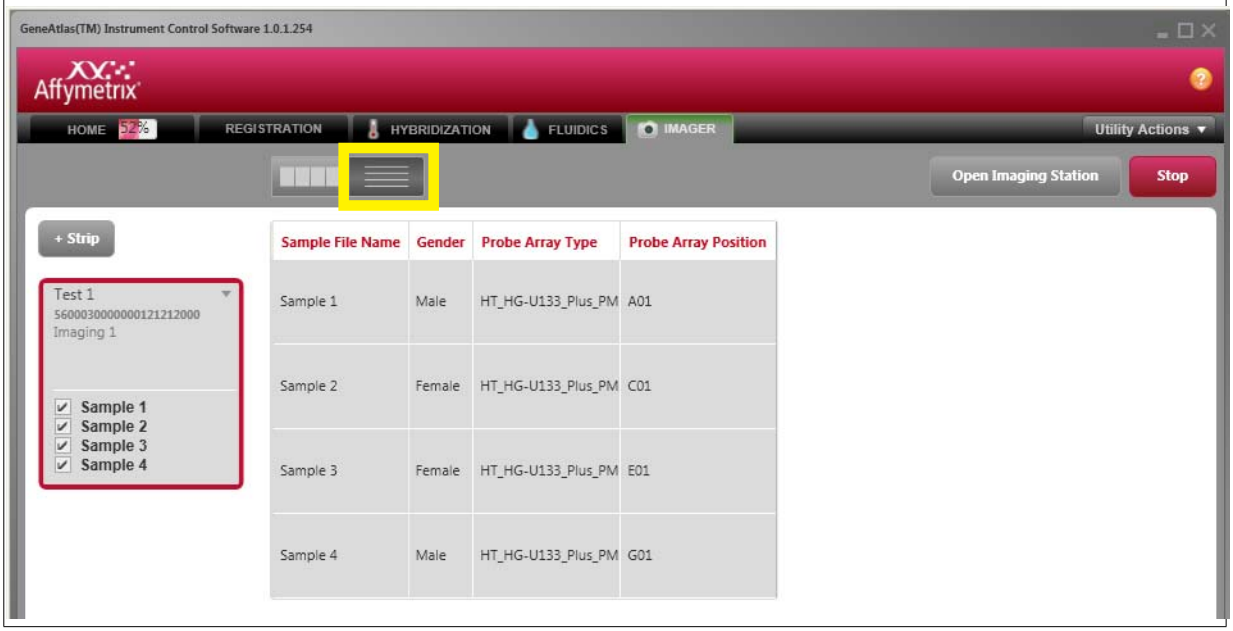
Green check marks appear next to the array names that have passed the internal QC parameters. If an array fails internal QC parameters, a red x mark and View Image link appears next to the array name (Figure 6.13).

Figure 6.13 Failed array scan

Click the View Image link to view the array in the Image Viewer.

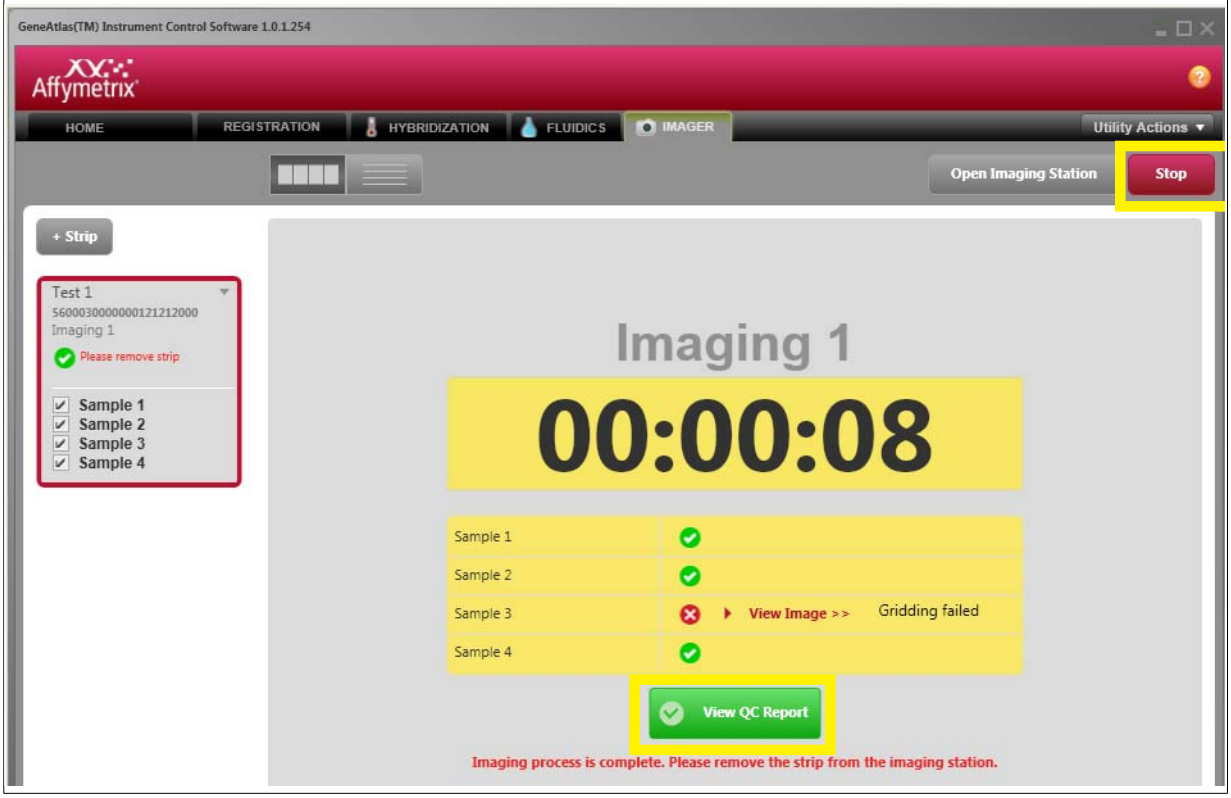
See a list of the arrays being imaged in the List view (Figure 6.14).

Figure 6.14 Imager tab, list view



A notice appears when imaging is finished and the QC Report button appears (Figure 6.15).

Figure 6.15 Imaging finished



You can click on the **View QC Report** to see a QC report on the arrays (see *Viewing a QC Report* on page 74).

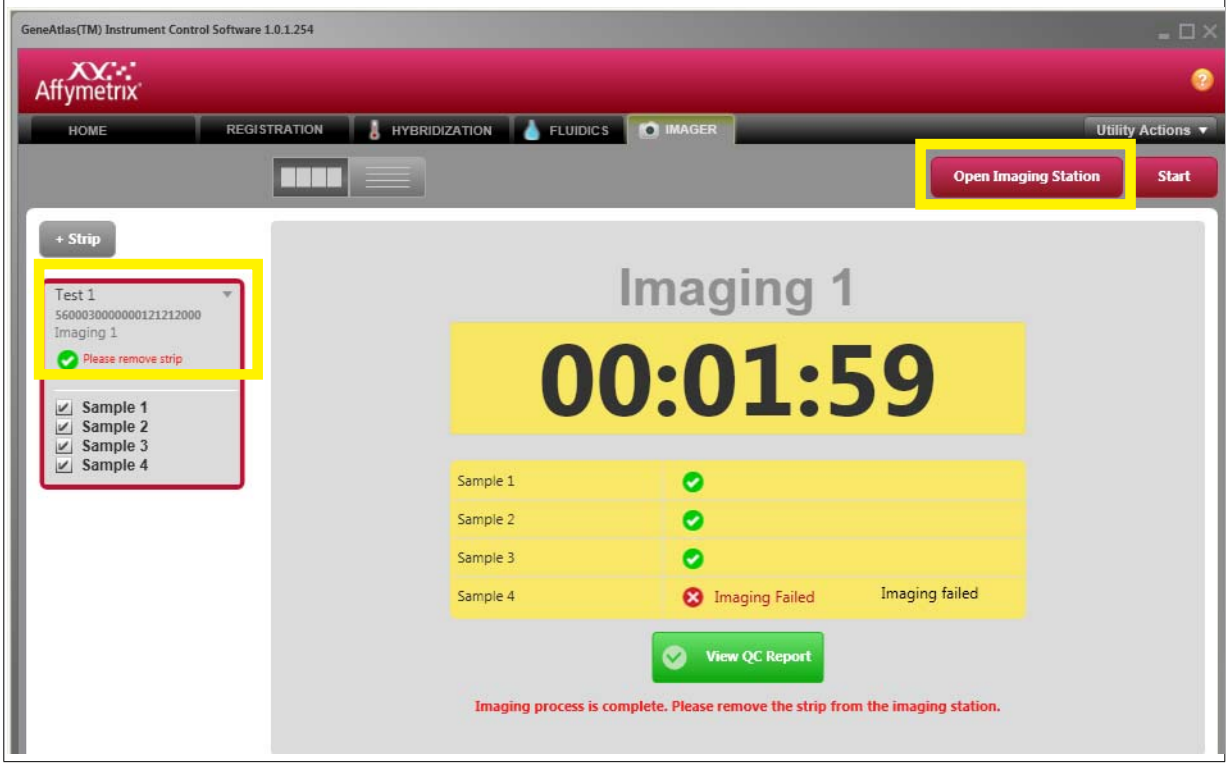
12. Click on the **Stop** button.
13. The Confirm Stop dialog box opens (Figure 6.16).

Figure 6.16 Sure to stop notice

14. Click **Yes.**

    The Open Imaging Station button appears in the Imaging tab (Figure 6.17).

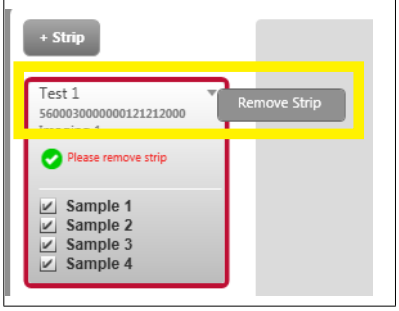
Figure 6.17 Imaging completed



15. Click **Open Imaging Station** and remove the array strip from the imager.
16. Click **Close Imaging Station**.

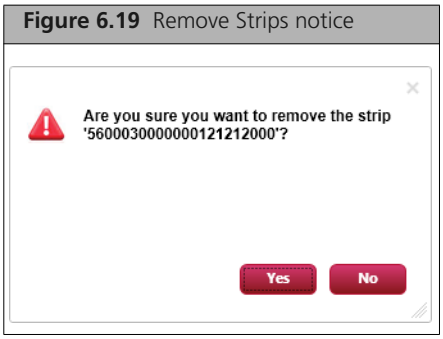
    The Imaging Station door closes.
17. Click on the dropdown arrow in the Strip box on the left of the screen and select **Remove Strip** from the menu.

Figure 6.18 Remove Strip menu



The Remove Strip Notice appears (Figure 6.19).

Figure 6.19 Remove Strips notice



18. Click **Yes**.

The array strip is removed from the system.

GeneAtlas® Utilities

The GeneAtlas Utilities provide the functions shown in Table 7.1.

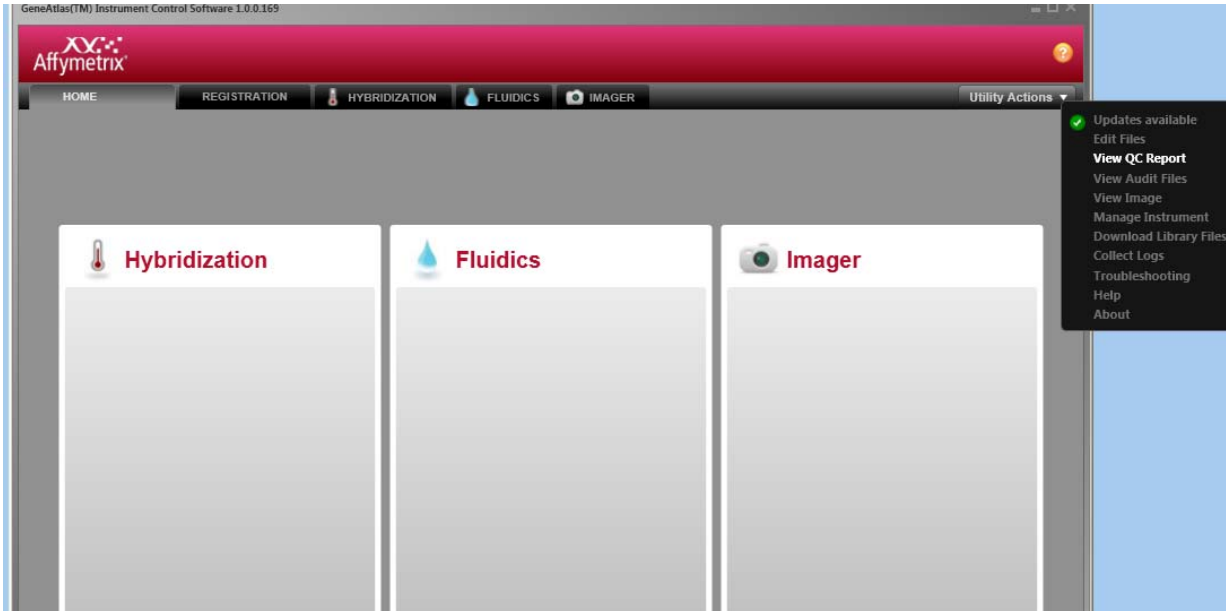
Table 7.1 The GeneAtlas utilities

Utility	Function	See
Updates Available	Notifies you of updates for GeneAtlas software that can be downloaded at affymetrix.com.	Links to the Affymetrix Update Page for the software.
Edit Files	Enables you to select and edit sample files (ARR).	*Editing Sample Files* on page 31
View QC Report	Shows the QC Summary Report for one or more user-selected samples.	*Viewing a QC Report* on page 74
View Audit Files	Shows the audit file for a user-selected sample.	*Viewing Audit Files* on page 80
View Image	Shows the intensity data (CEL) for a user-selected sample.	Appendix A, *Using the Viewer* on page 87
Manage Instrument	Enables qualification of the Fluidics Station or Imager Station and displays the qualification information.	*Select and Name Instruments* on page 19 of the *GeneAtlas Setup and Verification Manual*.
Download Library Files	Click to select and download library files from affymetrix.com.	*Download Library Files* on page 82
Collect Logs	Creates a system log of all activity on the GeneAtlas system, including a log files for the Fluidics Station and Imager Station.	*Collect Logs* on page 84
Troubleshooting	Not available	
Help	Opens the GeneAtlas Online Help.	
About	Opens the GeneAtlas About screen with information on the software.	

To start a utility:

1. Click **Utility Actions**.
2. Make a selection from the drop-down list.

Figure 7.1 Utilities Actions menu



Viewing a QC Report

To help researchers establish quality control processes for gene expression analyses, Affymetrix has developed several controls which enable researchers to monitor assay data quality. These include but are not limited to:

- hybridization controls
- labeling controls
- internal control genes

The QC report displays the results of these controls.

When analysis results are viewed within the software, the metrics for the report controls are compared to a set of pre-defined thresholds. Any results identified as being outside of the selected thresholds are tagged as Exclude.

In general, Affymetrix highly encourages users to create a running log of these parameters to monitor quality and potentially flag outlier samples. The QC functionality built into the GeneAtlas Software can help with this. Evaluation of particular samples should be based on the examination of all sample and array performance metrics in light of the history of the metrics performance in an individual tissue and array type.

Prior to excluding samples from downstream analysis, users should consult the Affymetrix troubleshooting website for potential reasons the controls may have failed and possible resolutions for the issue. For example, if hybridization controls are not spiked into the cocktail, then obviously the controls will fail, while the rest of the array data may be perfectly fine.

The QC Report displays information on:

- *Signal Value*
- *Hybridization Controls*
- *Labeling Controls*
- *Sample Quality (Housekeeping Genes)*

The features of the QC Report are described in the following sections:

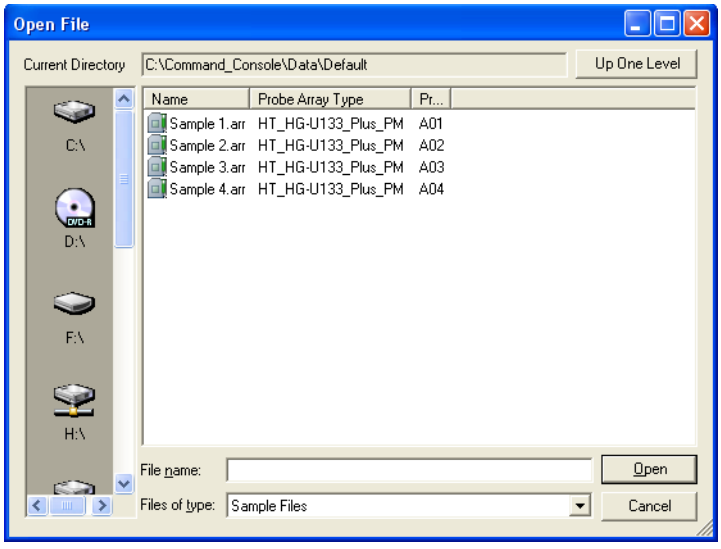
- *Opening a QC Report*
- *Summary View* on page 76

Opening a QC Report

To open a QC Report:

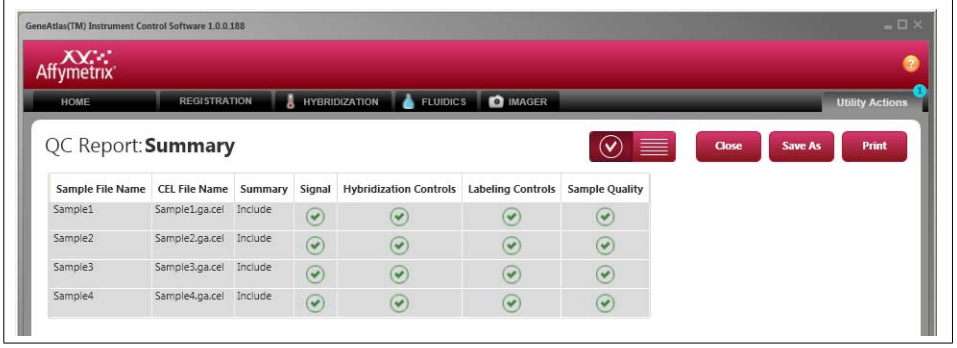
1. From the Utility Actions menu, select **View QC Report**.
   The Open dialog box appears (Figure 7.2).

Figure 7.2 Open dialog box



2. Select the file(s) to open and click **Open**.
   The QC report is displayed (Figure 7.3).

Figure 7.3 QC Report: Summary view



Buttons at top of the QC Report allow you to:

Show the Summary view for a quick check of the QC status (see *Summary View*, below).

Show the Detail view that includes additional information to help you evaluate the quality of your results and locate problems (see *Detail View* on page 77).

Close the QC report.

Save the QC report (.txt).

Print the QC report.

Summary View

The Summary view displays the basic pass-fail status of the arrays (Figure 7.4).

Figure 7.4 QC Report: Summary view

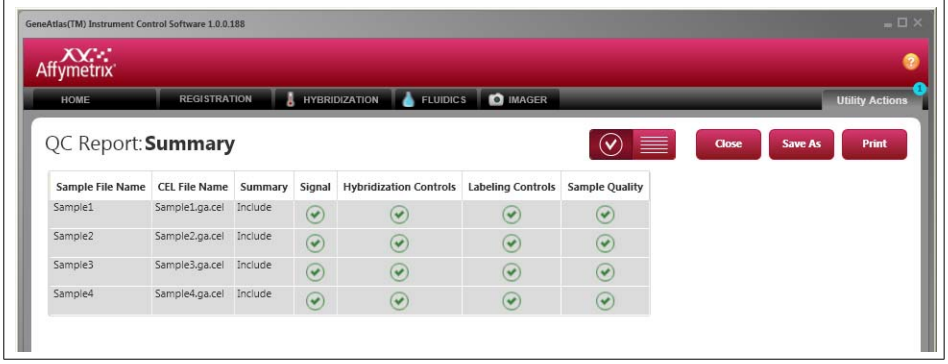


Table 7.2 QC Report, Summary view

Item	Description
ARR File Name	Sample name
CEL File Name	File name automatically generated by the system for the intensity data
Summary	Include - Sample data meets QC thresholds. Exclude - Sample data does not meet QC thresholds. It is recommended that you exclude the sample from downstream analysis.
Signal	- Intensity data (CEL) meet QC thresholds. - Intensity data do not meet QC thresholds.
Hybridization Controls Labeling Controls	- Control data meet QC thresholds. - Control data do not meet QC thresholds.
Sample Quality	- GAPDH meets sample quality thresholds. - GAPDH does not meet sample quality thresholds.

Detail View

The Detail View provides more information about the QC metrics. This information can help you evaluate problems if they arise (Figure 7.5).

Figure 7.5 QC Report: Detail view

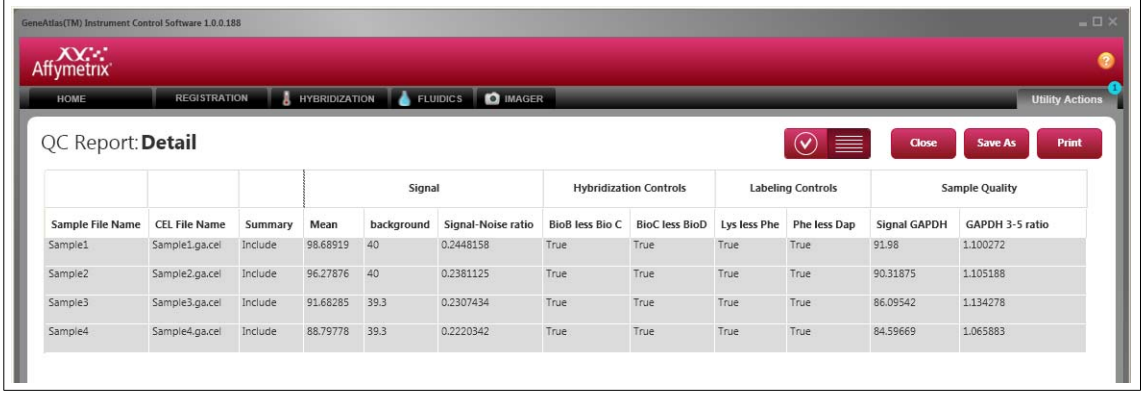


Table 7.3 QC Report, Detail view

Item	Description
ARR File Name	Sample file name
CEL File Name	Intensity data file name
Summary	- Labeling, hybridization, and sample quality controls meet QC thresholds. - A labeling, hybridization, or sample quality control does not meet QC threshold.
Signal (Mean)	The average signal of the probesets on the array (after removing background signal).
Signal (background)	The average value of the background signal
Signal (signal-noise ratio)	Signal to noise ratio for the array: (log2 mean signal - log2 background)/log2 background
Hybridization Controls	BioB less BioC - If False, the table cell for the array and item is highlighted in red. BioC less BioD - If False, the table cell for the array and item is highlighted in red. If both thresholds are not met, the hybridization controls are marked in the summary report.
Labeling Controls	Lys less Phe - If False, the table cell for the array and item is highlighted in red. Phe less Dap - If False, the table cell for the array and item is highlighted in red. If both thresholds are not met, the labeling controls are marked in the summary report.
Sample Quality	Signal GAPDH - Intensity signal for GAPDH GAPDH 3-5 ratio - The ratio of 3′ GAPDH probe set signals to 5′ GAPDH probe set signals. - Ratio < 3. - Ratio is ≥ 3.

The QC Parameters

The QC Report displays information on:

- *Signal Value*
- *Hybridization Controls*

- *Labeling Controls*
- *Sample Quality (Housekeeping Genes)*

Signal Value

This is a measure of the average brightness of the probe sets on the array, minus the background noise. The Signal to Noise ratio must be above a certain value for the array to pass QC.

Hybridization Controls

Biotin labeled controls added to hybridization cocktail.

The 20X Eukaryotic Hybridization controls are high-quality controls for monitoring array hybridization, washing, and staining for reproducible results.

The 20X Eukaryotic Hybridization Controls are composed of a mixture of biotinylated and fragmented aRNA of *bioB*, *bioC*, and *bioD* from *E. coli* in staggered concentrations. The premixed controls are ready to be added directly to the hybridization cocktail.

The 20X Eukaryotic Hybridization Controls are spiked into the hybridization cocktail, independent of RNA sample preparation, and are thus used to evaluate sample hybridization efficiency on eukaryotic gene expression arrays.

Labeling Controls

Poly A Controls are added to RNA Sample prior to using IVT express kit.

Four independent poly-A RNA controls, derived from the l*ys*, *phe*, and *dap* genes of *B. subtilis*, are provided conveniently in a pre-mixed stock solution at staggered concentrations. After spiking directly into eukaryotic total RNA samples, labeled aRNA targets are prepared and hybridized onto GeneChip expression arrays. The resultant signal intensities for the poly-A RNA controls serve as sensitive indicators of the efficiency of the labeling reaction and are independent of input sample RNA quality.

Sample Quality (Housekeeping Genes)

Housekeeping genes are gene transcripts that are constitutively expressed on most samples. These transcripts serve as internal controls, are useful for monitoring the quality of the starting sample, and are subject to any variability in the labeling of the sample and hybridization for the array. For Human, Mouse, and Rat 3' expression arrays, GAPDH is used to assess RNA sample and assay quality. The signal values for the 3' probe sets are compared to the signal values for the corresponding 5' probe sets. If the ratio is greater than 3, the sample is failed.

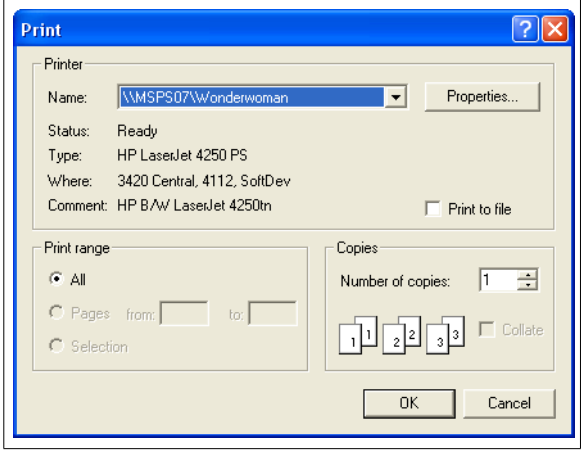
Printing the QC Report

NOTE: The Print function only prints the content displayed on the QC Report screen at the time (Summary or Detail).

To print out the QC Report:

1. Click the **Print** button.
   The Print dialog box opens (Figure 7.6)

Figure 7.6 Print dialog box.



2. Select a printer and other options and click **OK**.
   The report is printed.

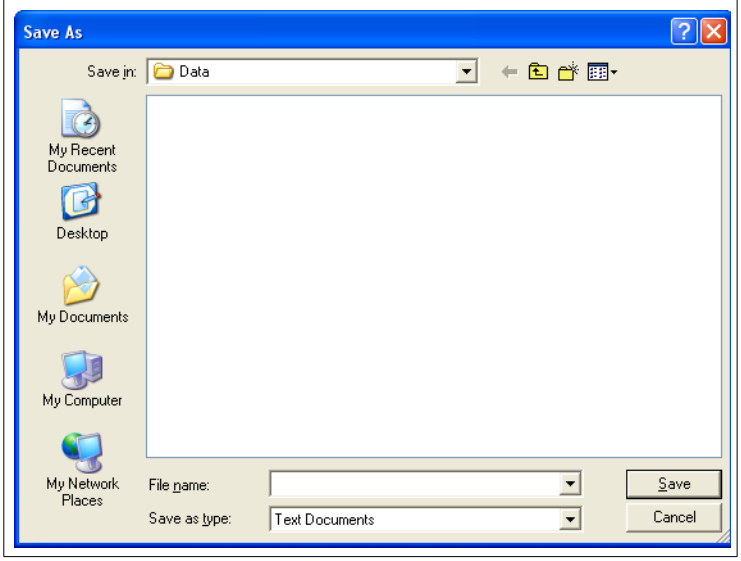
Saving the QC Report

NOTE: The Print function only saves the content displayed on the QC Report screen at the time (Summary or Detail).

To save the QC Report:

1. Click the **Save** button.
   The Print dialog box opens (Figure 7.6)

Figure 7.7 Print dialog box.



2. Enter a file name for the file and click **Yes**.
   The report is saved in a text format file.

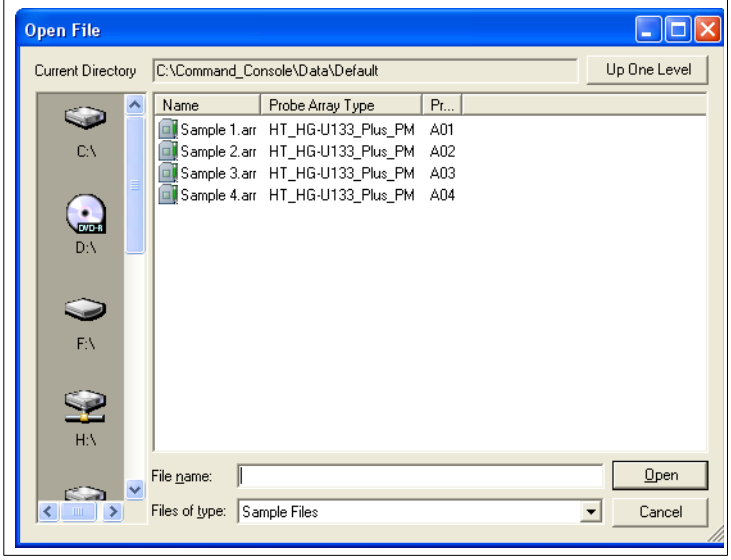
Viewing Audit Files

An audit file (Figure 7.9) displays information about the:

- Array
- Hybridization
- Fluidics processing
- Imaging

1. From the Utility Actions menu, select **View Audit Files**.
   The Open dialog box appears.

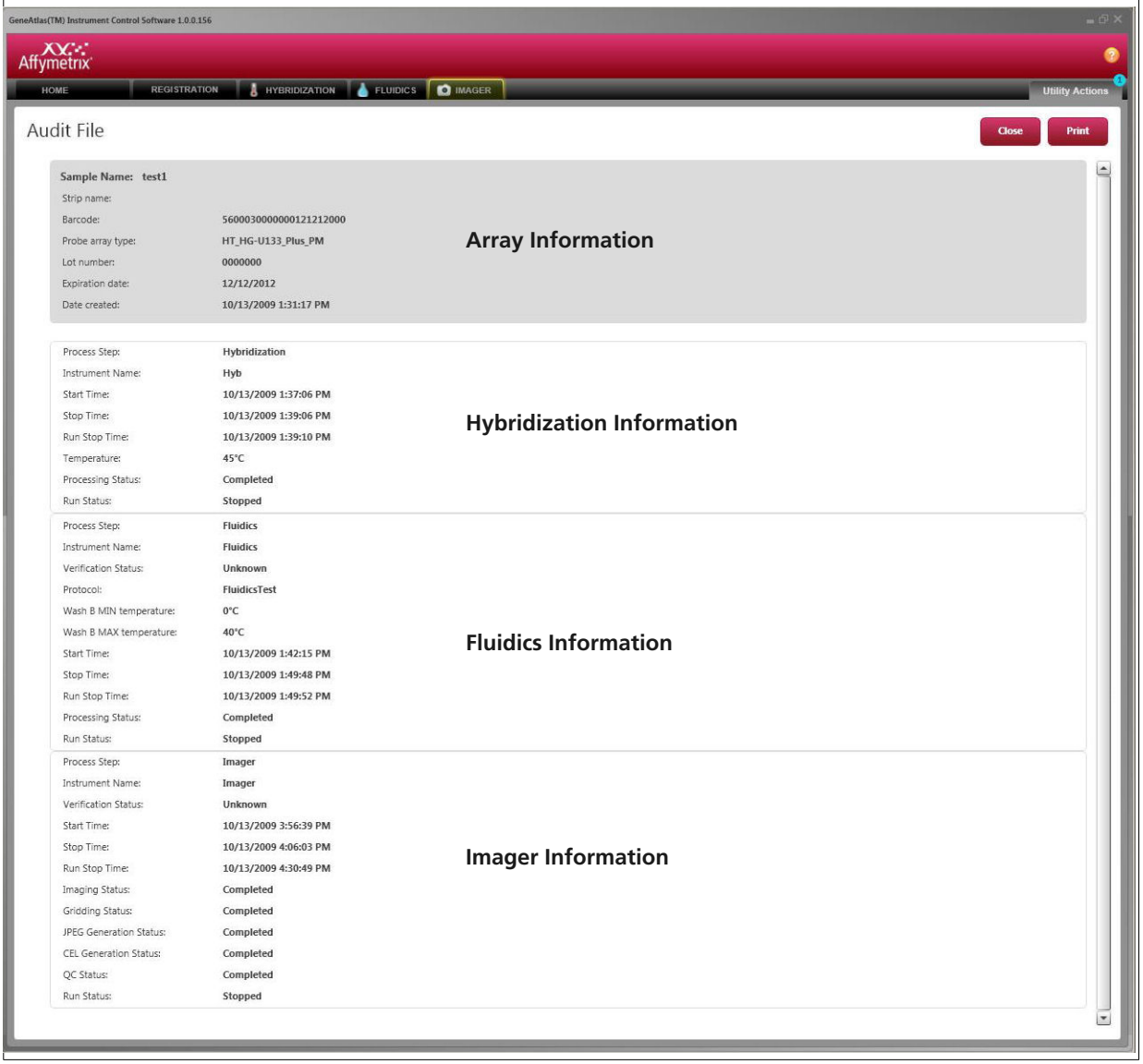
Figure 7.8 Open File dialog box for audit files



2. Select the sample file that you want to view the audit file for and click **Open**.
   The audit file is displayed (Figure 7.9).
   The file displays information on all of the array processing workflow steps that have been started:
   - *Array Information* on page 81
   - *Hybridization Information* on page 82
   - *Fluidics* on page 82
   - *Imager Information* on page 82

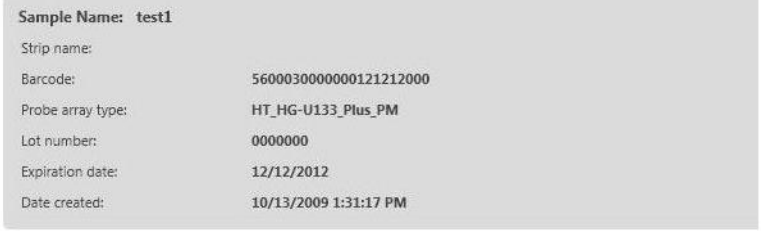
   If a workflow process has not been started, the information will not be displayed in the Audit file.
   If a workflow process has been run more than one time, the information will be displayed separately.

Figure 7.9 View Audit File window



Array Information

Figure 7.10 Array Information

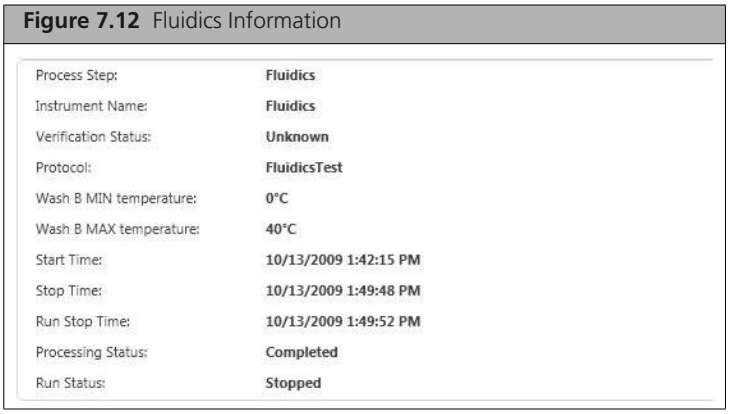


Hybridization Information

Figure 7.11 Hybridization Information

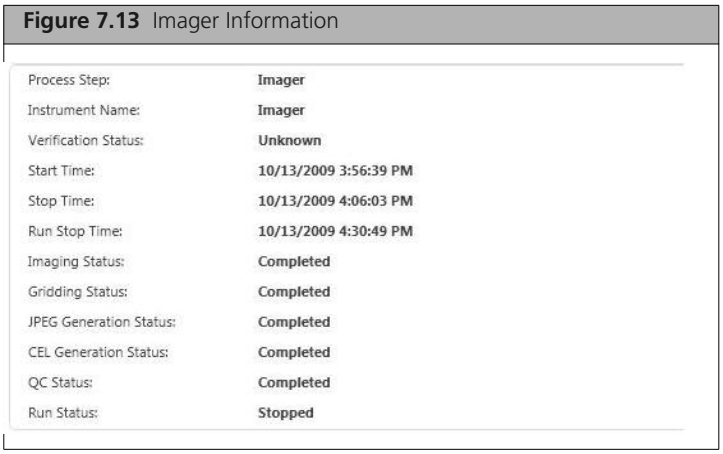
Fluidics

Figure 7.12 Fluidics Information



Imager Information

Figure 7.13 Imager Information



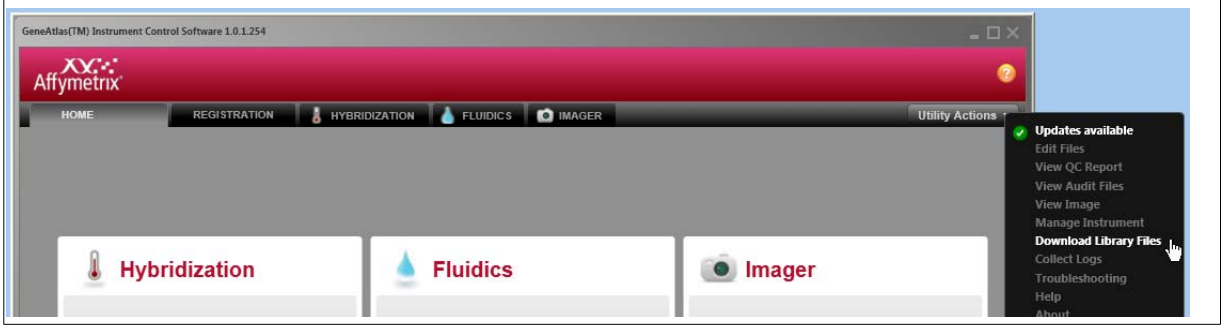
Download Library Files

The Download Library Files function lets you download the latest library files from the NetAffx website. You will need to create a NetAffx account if you have not already done so.

To download libraries:

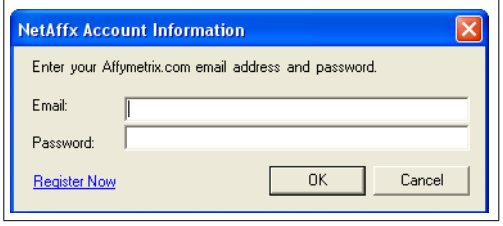
1. From the Utility Actions menu, select **Download Library Files** (Figure 7.14).

Figure 7.14 Utility Actions menu



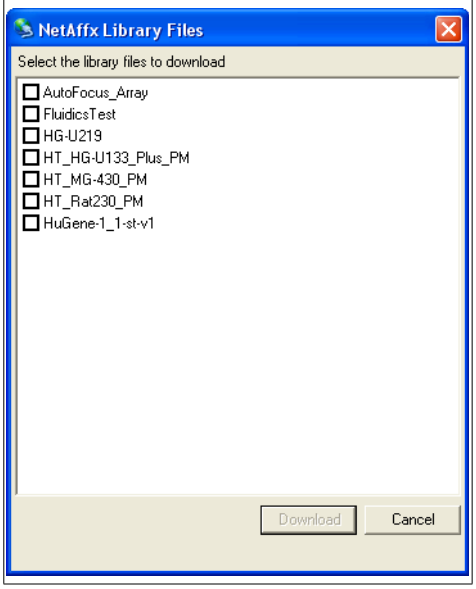
The NetAffx Account Information dialog box opens (Figure 7.15).

Figure 7.15 NetAffx Account Information Dialog Box



2. If you have not created a NetAffx account, click the **Register Now** link to do so.
3. If you have created a NetAffx account, enter your email and password and click the **OK** button. The NetAffx Library Files dialog box opens (Figure 7.16).

Figure 7.16 NetAffx Library Files dialog box



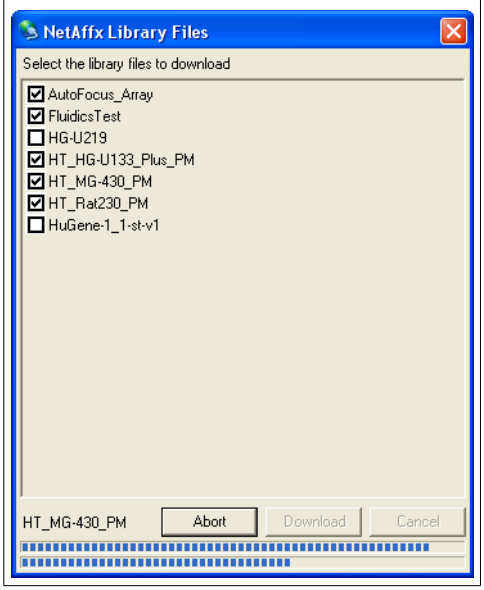
The list includes:

- Files for Instrument Verification
  - AutoFocus Array

- FluidicsTest
- Files for different array types
  - HT_HG_U219_PM
  - HT_MG-430_PM
  - HT_Rat230_pm

4. Select the checkbox next to the libraries you wish to download.
5. Click the **Download** button
   The dialog box displays the progress of the download.

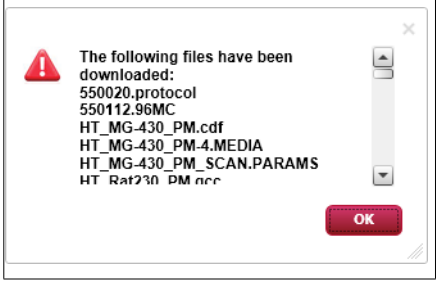
Figure 7.17 Download progress



Click the **Abort** button to halt the download.

When the download is halted or finished, a list of the downloaded library files is displayed (Figure 7.18).

Figure 7.18 Download complete list



6. Click **OK** to close the list.

Collect Logs

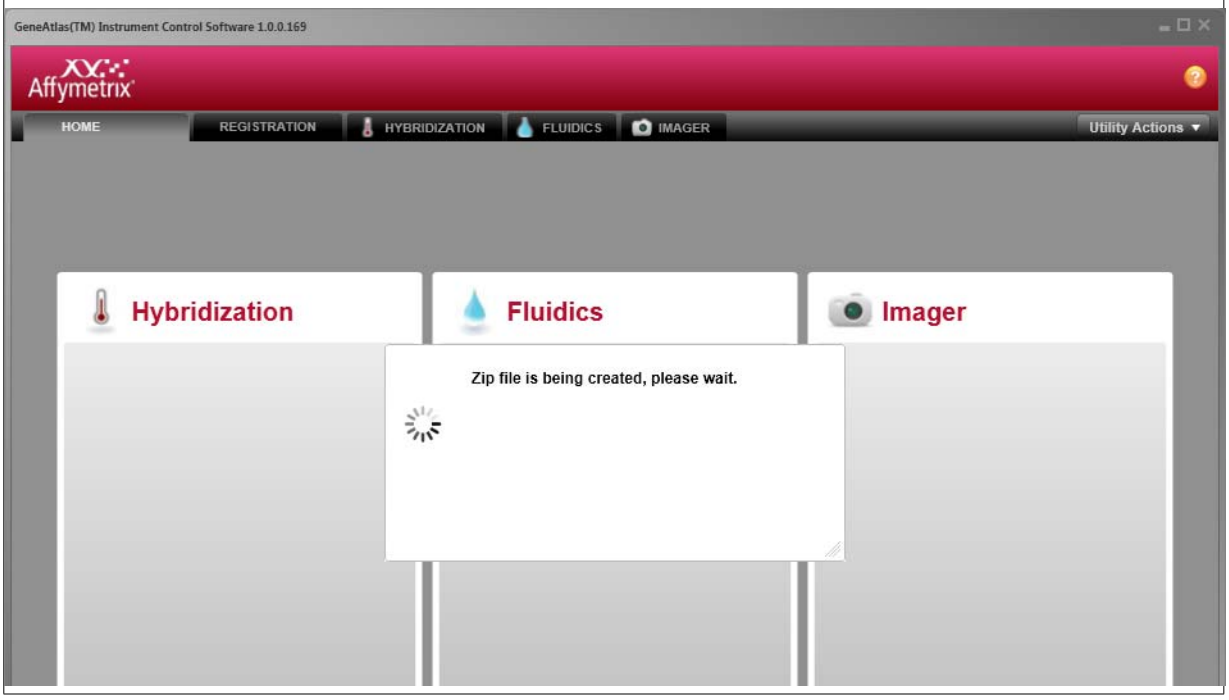
Log files are produced by the different GeneAtlas components. The logs provide a record of the tasks performed by the different components, such as the Fluidics and Imaging Stations and the installer. These log files may provide useful information for troubleshooting problems.

The Collect Logs utility creates a zip package of all the log files for the GeneAtlas System, including log files for the Fluidics Station and the Imaging Station. Upon request you can send the zip package to Affymetrix Support.

To create a zip package of the log files:

1. From the Utility Actions menu, select **Collect Logs**.
   A notice that the zip file is being created appears (Figure 7.19).

Figure 7.19 Zip file creation notice



When the file is complete, a notice (Figure 7.20) appears with the file name (based on the date and time of collection) and location.

Figure 7.20 Notice of zip file name and location

2. Click **OK** to close the notice.
   You can now send the file to Affymetrix Support.

Using the Viewer

After the array has been imaged, the GeneAtlas® System:

- Aligns a grid on the Image (DAT) file to identify the probe cells.
- Computes the probe cell intensity data for the array and creates a CEL file.

The GeneAtlas Viewer displays DAT, CEL, and JPG files and enables you to:

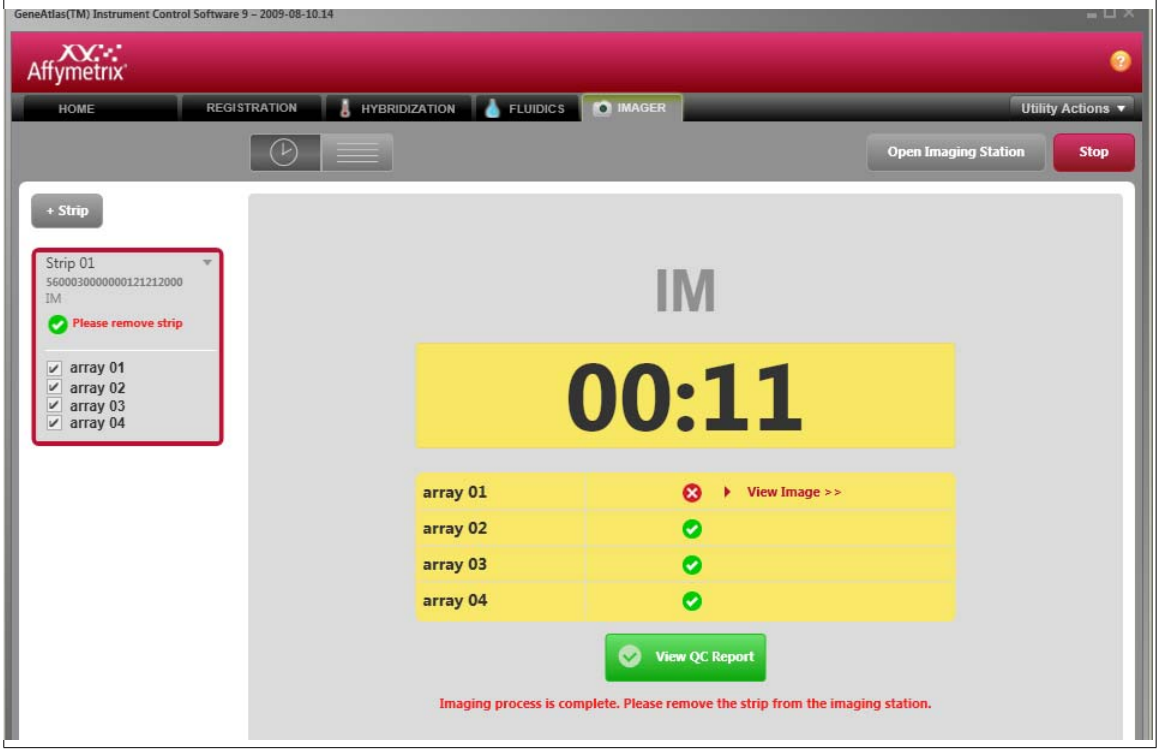
- View the files for quality control purposes.
- Perform grid alignment for sub images that have a gridding failure.
  See *Checking the Grid Alignment* on page 103.
- Create a JPG version of a DAT file for archiving.
  See *Exporting Images in Other Formats* on page 110.

This chapter describes the operation of the GeneAtlas Viewer in the following sections:

Opening the Image Viewer in the GeneAtlas® System

If an array fails to grid or generate a CEL file, a "view image" link is available on the User Interface. Clicking on the link will launch the image viewer.

Figure A.1 Imager Tab with View Image notice

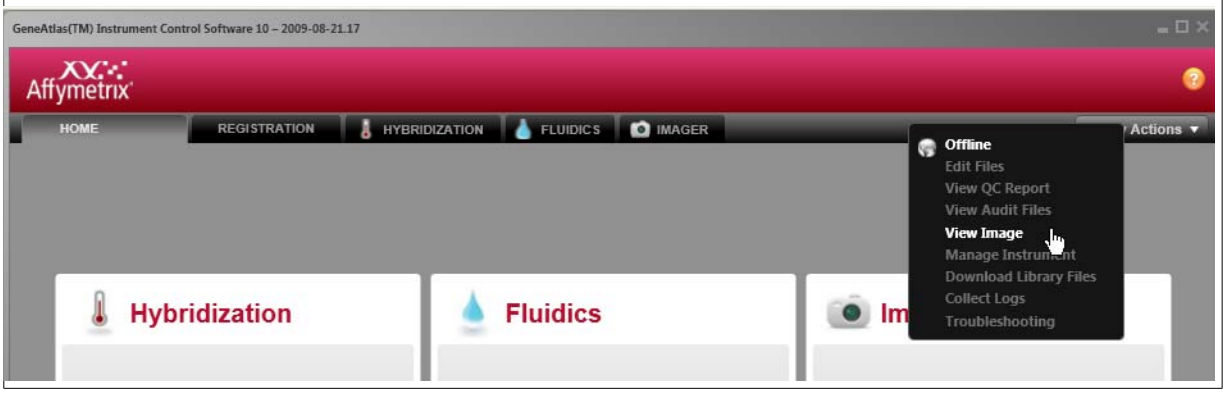


You can also open image files for inspection that did not fail gridding using the Open dialog box.

To open image files for inspection:

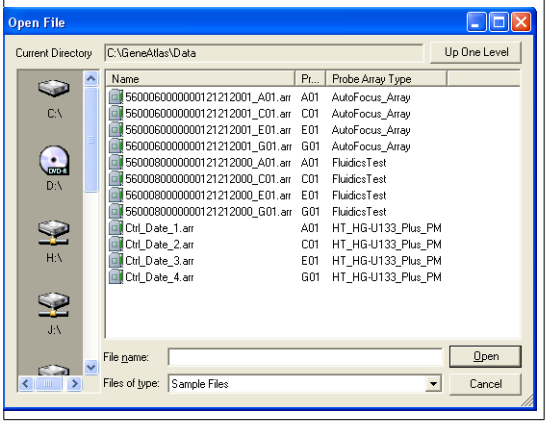
1. From the Utilities menu, select **View Image** (Figure A.2).

Figure A.2 Utilities Action menu



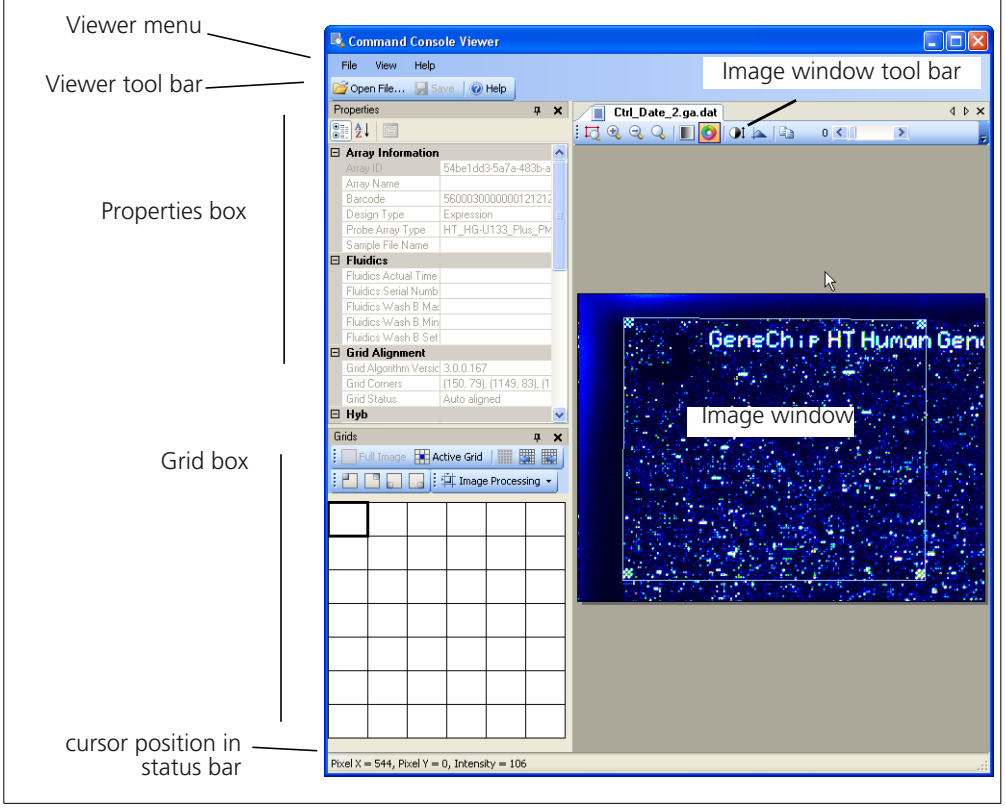
The Open dialog box opens (Figure A.3).

Figure A.3 Open dialog box



2. Select the array file that you wish to inspect the DAT file for and click **Open**. The Image Viewer opens with the DAT file for the selected array (Figure A.4).

Figure A.4 Parts of the GeneAtlas Viewer with DAT file displayed



The viewer has the following components when it first opens:

- Viewer menu
- Viewer tool bar
- Status bar: displays cursor position and intensity of selected pixel/cell

Additional components are visible when a DAT or CEL file is displayed.

To learn about the Image window tool bar, see *Changing the Display of the Image* on page 96.

To learn about the Properties box, see *Learning about the Image File* on page 102.

To learn about the Grid box, see *Checking the Grid Alignment* on page 103.

After opening the Viewer, you can use the Open dialog box to find and open other DAT and CEL files (see *Opening the Image Viewer in the GeneAtlas® System* on page 88)

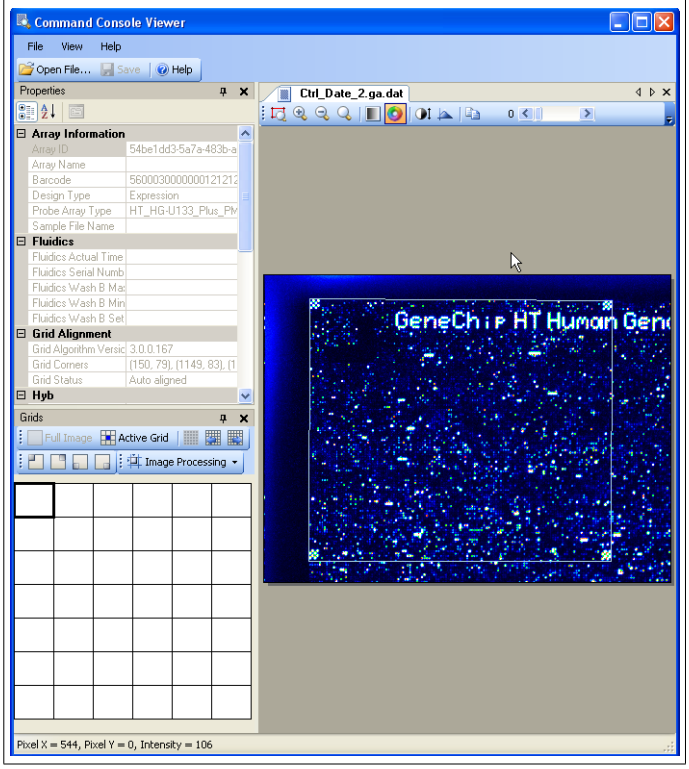
Array and Grid Types

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 image. The algorithm aligns the grid so that each square in the grid delineates a probe cell.

The alignment of the grids usually takes place automatically after imaging the array. If the alignment algorithm fails you can perform a manual alignment of the grids.

The GeneAtlas Arrays use grids (Figure A.5)

Figure A.5 GeneAtlas Array DAT file, grid selected

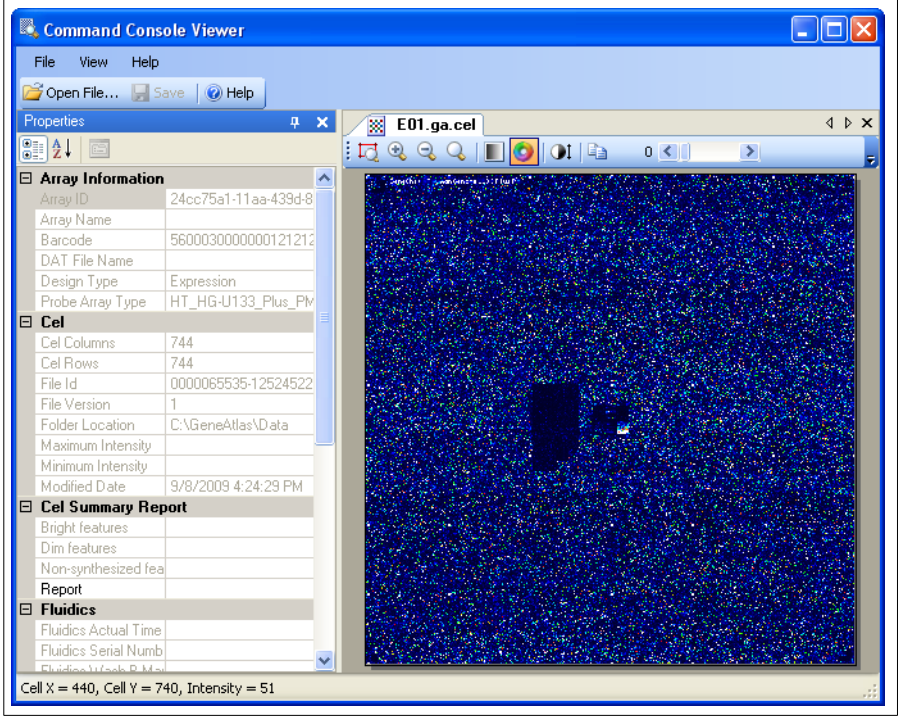


An array strip has four arrays.

Each array on the array strip has multiple grids. Some arrays use a 7 x 7 grid, for a total of 49 grids, while other array types use a 7 x 6 grid, for a total of 42 grids.

These sub-grids are similar to the sub-grids used for some cartridge arrays, but on an array strip array the sub-grids are not aligned to a main grid.

Figure A.6 GeneAtlas array (Full Image displayed as CEL file)



Each array is imaged twice, with different exposure times. Each image produces a single DAT file. This DAT file can be viewed in the Image Viewer; the file has all the image and gridding data for each grid and each exposure, and allows you to check the gridding independently for each exposure

The data from the DAT file is used to determine the cell intensity data for the grid for that exposure. Only one CEL file is produced per array. A CEL file is automatically generated after a DAT file is successfully gridded.

Introduction to the Viewer

You can learn more about the basic functions of the GeneAtlas Viewer in:

- *File Display Differences* on page 91
- *Moving the Components Out of the Viewer* on page 92
- *Moving the Component Borders in the GeneAtlas Viewer* on page 94

File Display Differences

The GeneAtlas Viewer has different types of functions and options for the different image file types that it displays:

DAT Files

DAT files are the image data files, the product of the initial imaging.They are used to generate the cell intensity data file after the grid has been aligned.

The DAT file must be opened to perform manual gridding or to run the grid alignment algorithm in the GeneAtlas Viewer.

If the cell intensity data (CEL) file has been generated, you can click the **Cell Intensity** button  and view the cell intensity data in the DAT Image window.

GeneAtlas DAT File Exposures

Each array is imaged twice, with different exposure times. The image data from both exposures are in the GeneAtlas Array DAT file. You can switch between the different exposures for checking the gridding, etc., by using the Exposure button (see *Displaying Different Exposures (GeneAtlas DAT Files Only)* on page 100).

CEL Files

CEL files are cell intensity data files, produced using the DAT file data after gridding and feature extraction.

You cannot perform grid alignment or cell generation on a CEL file.

JPG Files

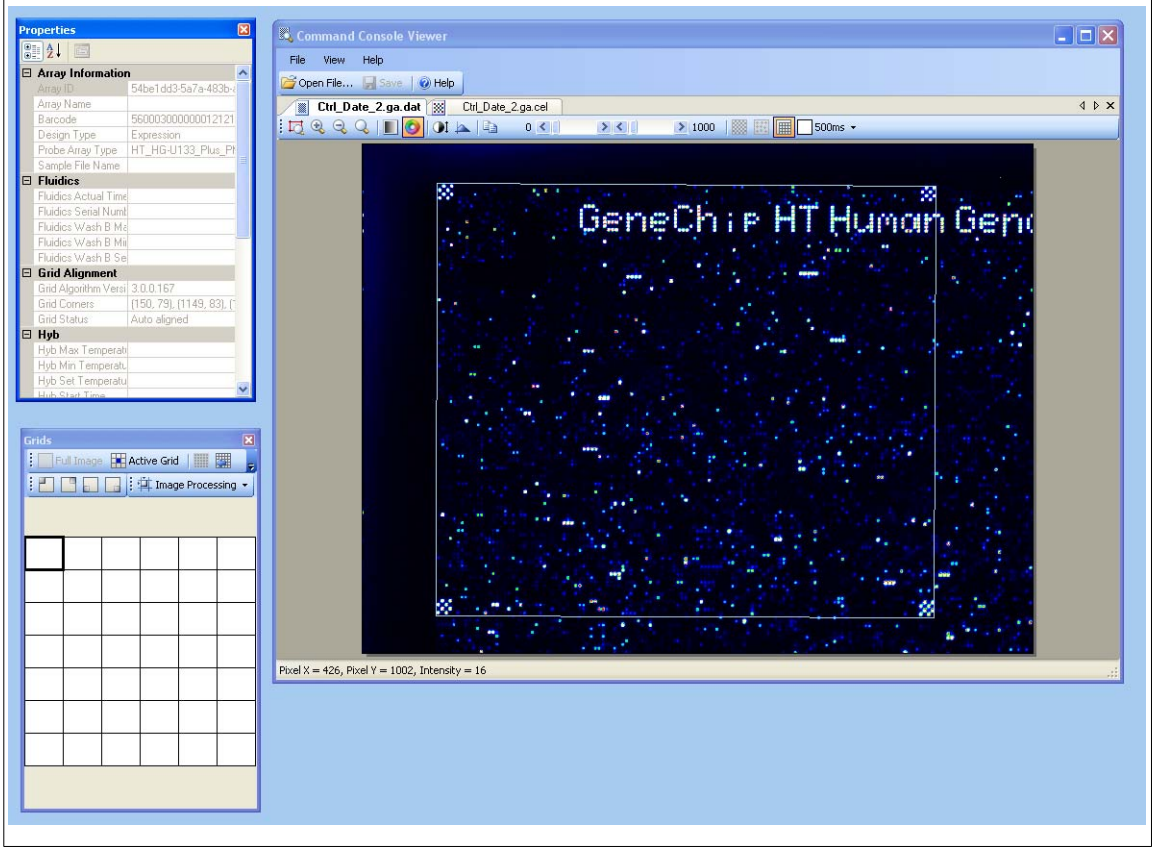
JPG files are a copy of the DAT image in a standard image file format; they provide an image file with a reduced file size for QC inspection, archiving, and publication.

Moving the Components Out of the Viewer

You can move the following components to a different location on your screen by clicking in the title bar and dragging the box to the new location (Figure A.7).

- Properties box
- Grid box

Figure A.7 Dragging boxes outside of the GeneAtlas Viewer



You can move the Image window outside of the Viewer by clicking on the file name tab and dragging the window out of the viewer.

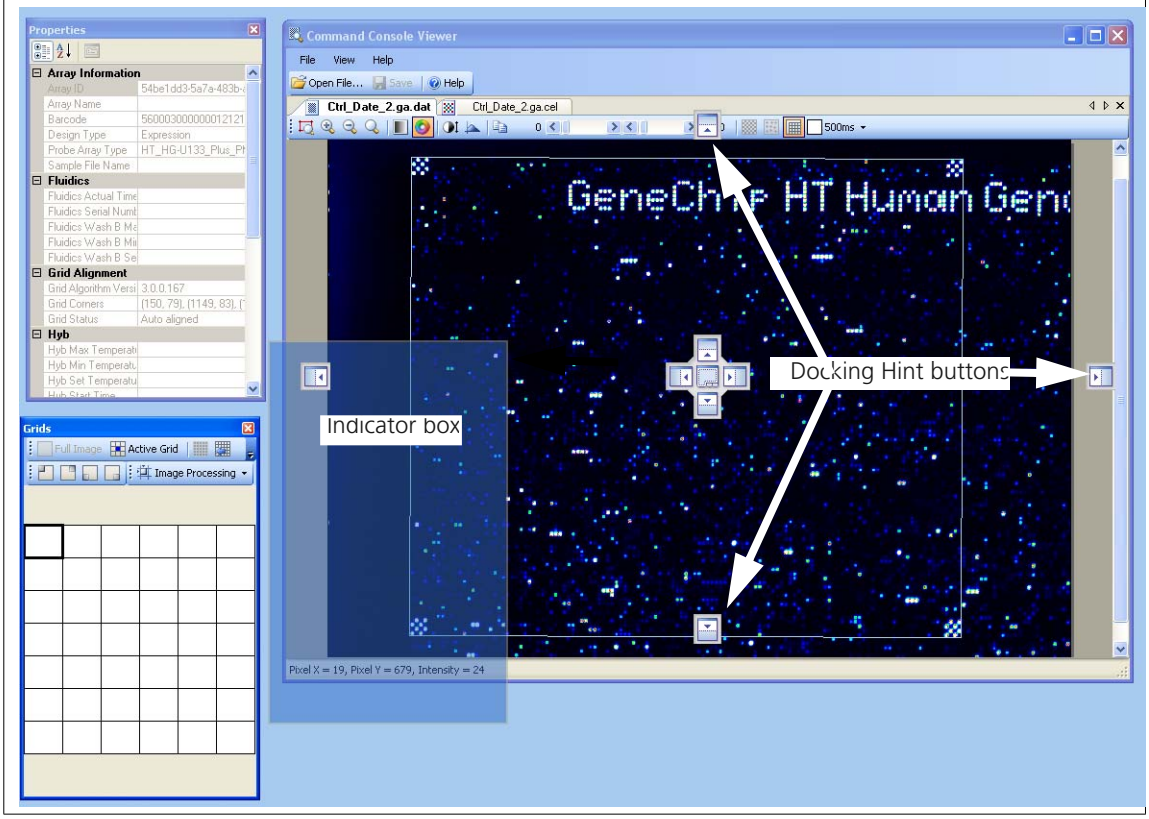
To dock the boxes back in the GeneAtlas Viewer:

- Double-click on the box title bar or the file name tab in the Image window.

To choose a new location for the box:

1. Click on the title bar and drag the box back into the GeneAtlas Viewer.
   The docking hints buttons appear in the Viewer (Figure A.8).

Figure A.8 Using the Docking Hints buttons



2. Move the Cursor to the docking hint button.
   A gray box appears to show where the box will dock.
3. Release the mouse button.
   The box is docked in the selected location.

The Box title bar contains some controls for the Properties and Grid boxes (Figure A.9):

Figure A.9 Box title bars

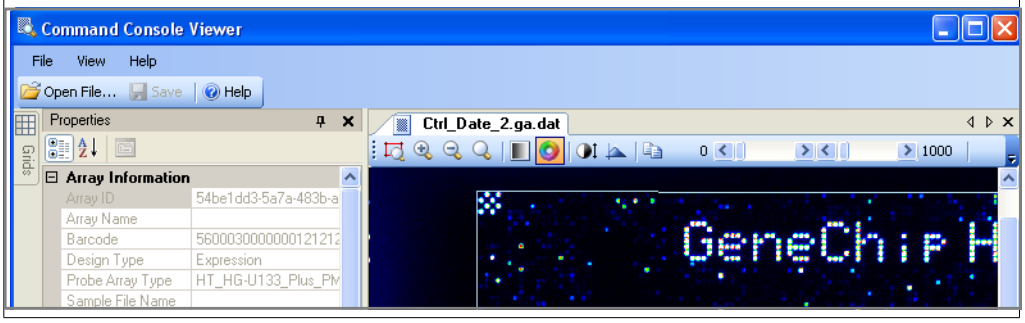
To close a box:

- Click the **Close** button

To use the Autohide feature:

- Click the **AutoHide** button in the box.
  The box is closed, and a tab is displayed on the left side of the window (Figure A.10).

Figure A.10 Hidden Grid Box and tab



To display a hidden box temporarily:

- Place your cursor on the tab.

To restore a hidden box:

- Display the box and click on the **AutoHide** button .

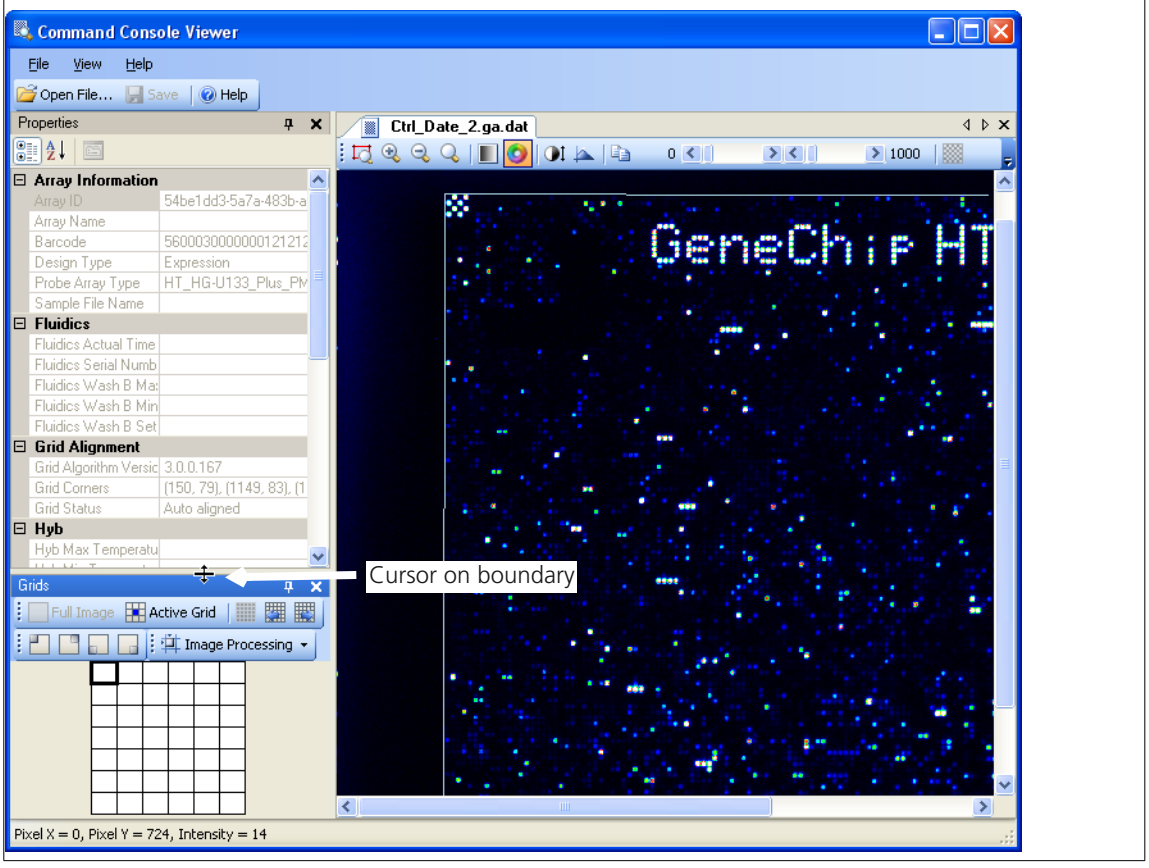
Moving the Component Borders in the GeneAtlas Viewer

You can change the relative size of a component in the Viewer by moving the borders of that component.

To change the size of the component:

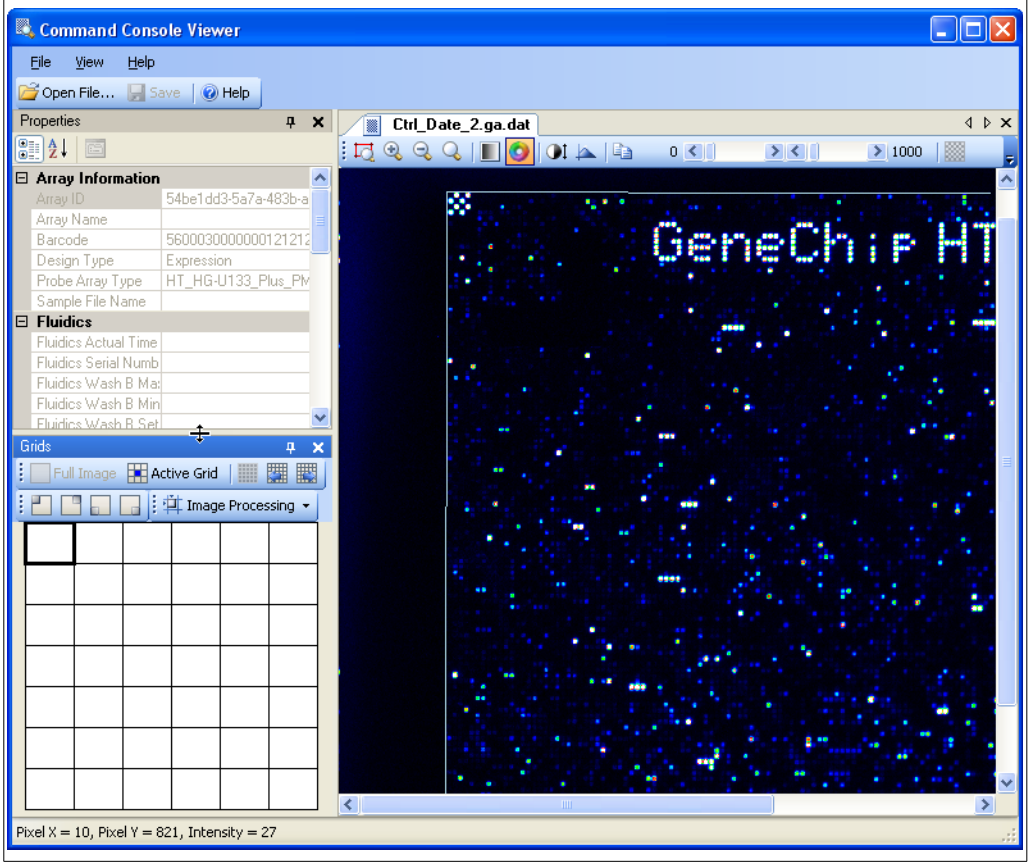
1. Move the cursor over the border until it changes to a double arrow or .

Figure A.11 Placing cursor on boundary between two components



2. Click and drag the cursor to change the size of the area (Figure A.12).

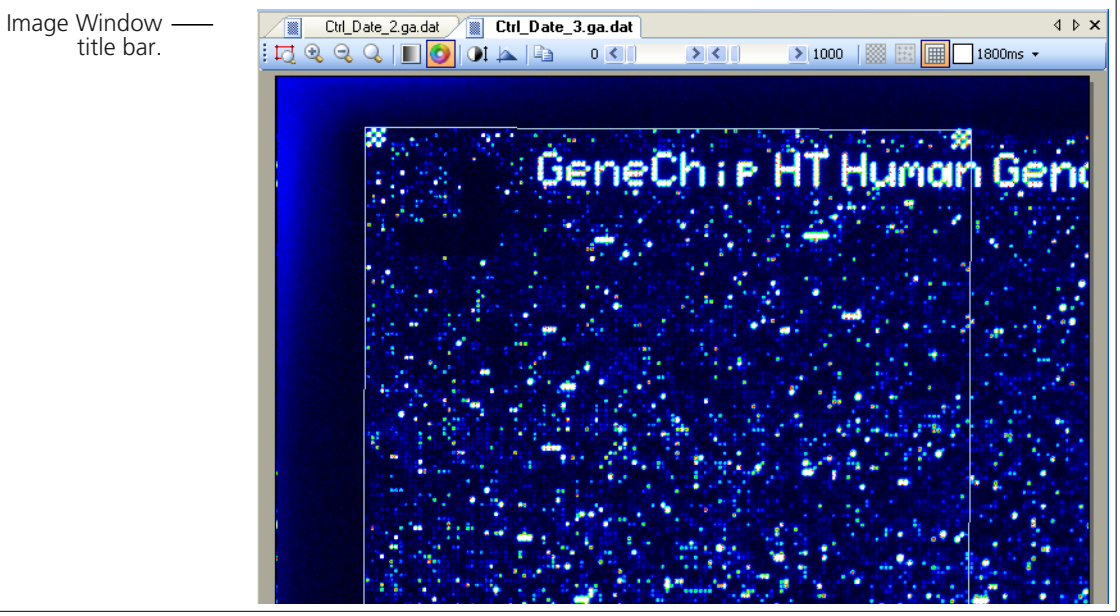
Figure A.12 Grid box enlarged.



Displaying Multiple Files

You can open more than one file in the GeneAtlas Viewer (Figure A.13).

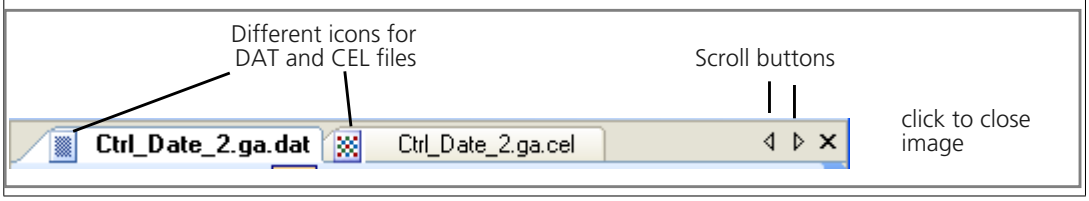
Figure A.13 Displaying multiple image files



To display a particular image when you have more than one open:

- Click the tab at the top of the Image Window.
  Use the < and > scroll buttons in the Image title bar to scroll through the tabs if necessary (Figure A.14).

Figure A.14 Image window title bar

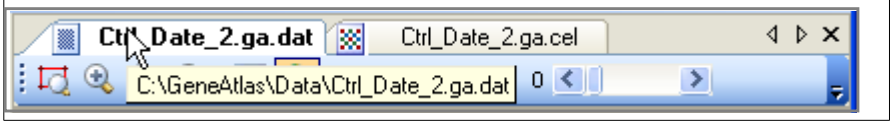


Different icons are used for DAT and CEL files.

To display the full path to a displayed file:

- Place your cursor on the file's title bar tab.
  The full path is displayed below the title bar (Figure A.15).

Figure A.15 Displaying the path to a file

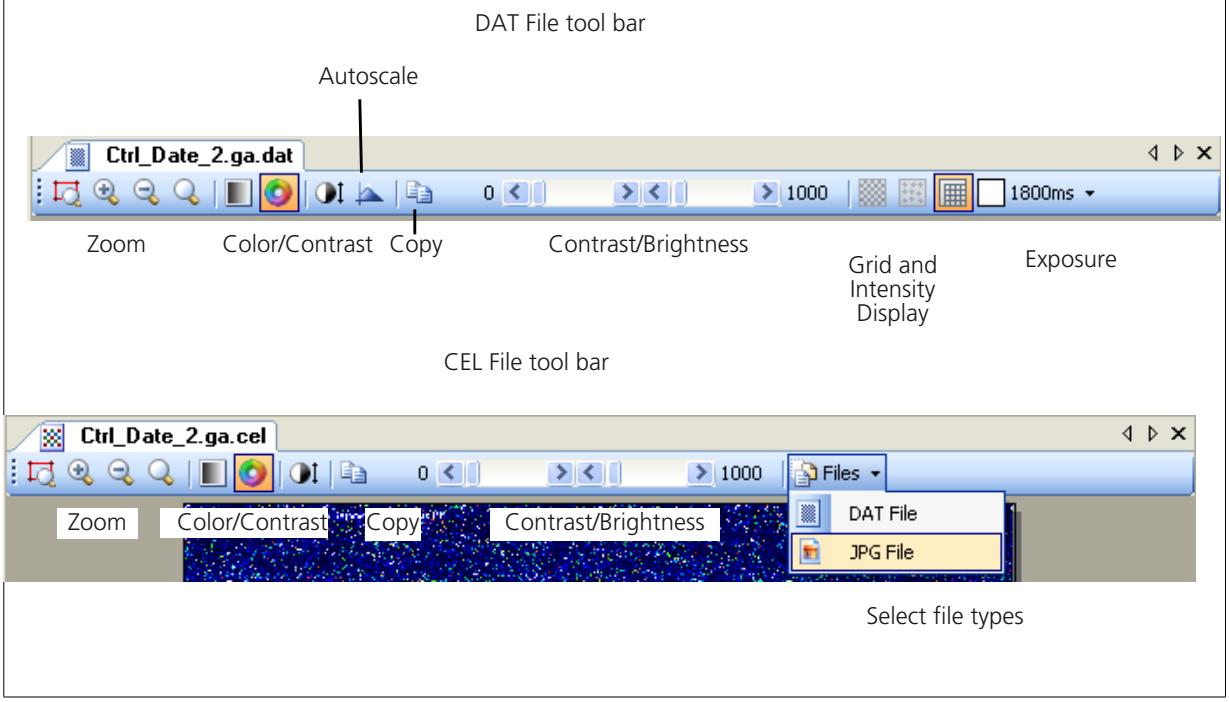


Changing the Display of the Image

This section explains how to use the Image tool bar controls (Figure A.16) for:

- *Examining Different Parts of the Image* on page 97
- *Adjusting the Colors and Contrast* on page 99
- *Changing the Grid and Intensity Display* on page 100

Figure A.16 Image window tool bar for DAT and CEL file

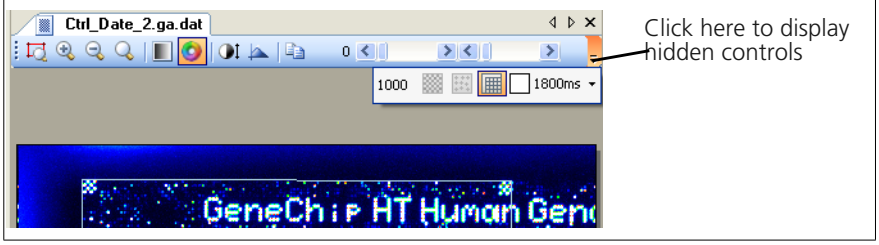


Part of the tool bar may be hidden if the GeneAtlas Viewer is too small.

To display the hidden controls:

- Click on the **Hidden Tool Bar** button at the right of the toolbar (Figure A.17).

Figure A.17 Displaying hidden controls



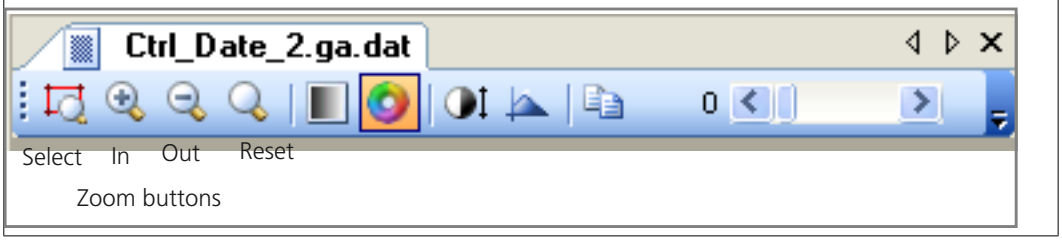
The hidden controls are displayed below the tool bar (Figure A.17).

Examining Different Parts of the Image

These functions work on DAT, CEL, and JPG files.

The Zoom controls are at the left end of the tool bar.

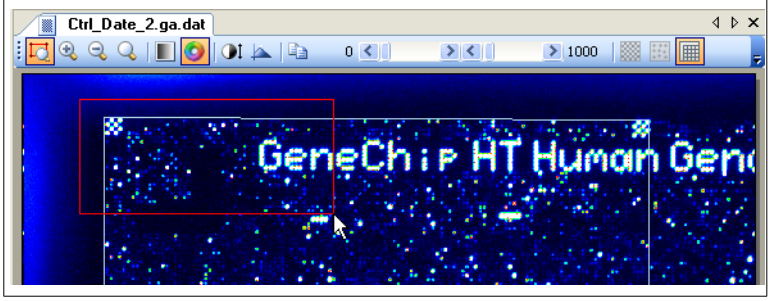
Figure A.18 Zoom controls



To zoom in on a selected area of the image:

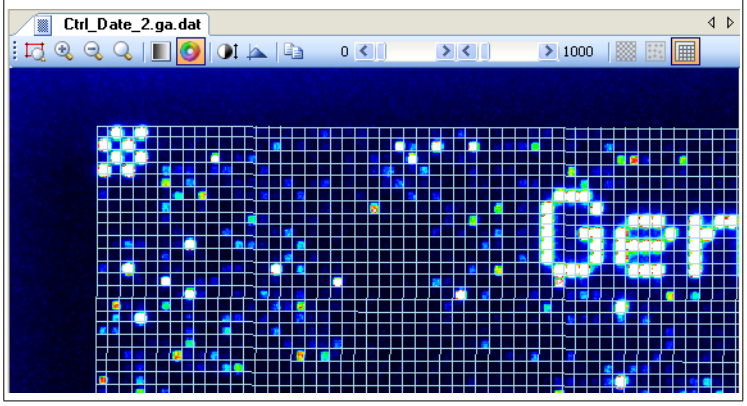
1. Click on the **Zoom Select** button .
2. Click and drag around the area you want to examine in more detail (Figure A.19).

Figure A.19 Click and drag



3. Release the mouse button.
4. The selected area is displayed in the GeneAtlas Viewer (Figure A.20).

Figure A.20 Zoomed-in view (with grid displayed)



To zoom in or out on the whole image:

- Click on the Zoom In button or the Zoom Out button .

To view a different area in magnified zoom:

- Click and drag the image to view the area of interest.

To zoom out:

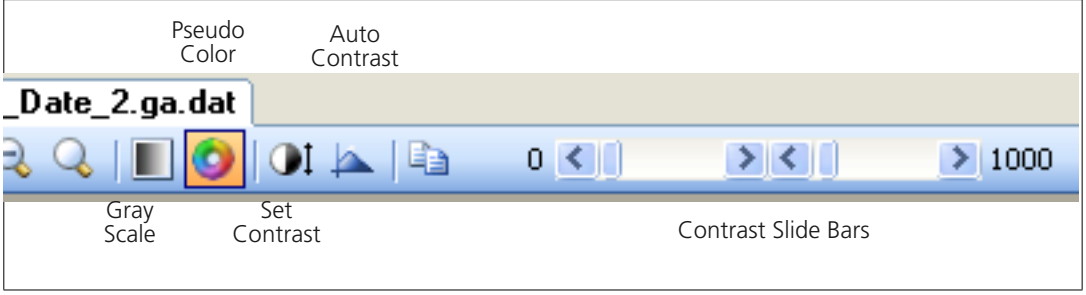
- Click the **Zoom Reset** button .

NOTE: You can also use the Grid box controls to select a particular corner or grid for examination (see *Checking the Grid Alignment* on page 103).

Adjusting the Colors and Contrast

These functions work on DAT, CEL, and JPG files.

Figure A.21 Color and Contrast controls



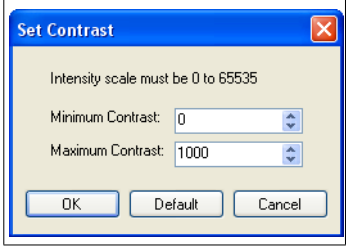
To switch between Gray Scale or Pseudo Color display:

- Click the **Gray Scale** or **Pseudo Color** buttons.

To adjust the contrast range for the image:

1. Click the **Set Contrast** button.
   The Set Contrast dialog box opens (Figure A.22).

Figure A.22 Set Contrast dialog box



2. Set the minimum and maximum contrast range.
3. Click **OK** to use the settings; or
   Click **Default** to return to the default settings; or
   Click **Cancel** to close the dialog box without changing the settings.

You can also use the slide bars in the tool bar (Figure A.21) to set the contrast without opening the Set Contrast dialog box.

Using the Autoscale Function

The autoscale function takes the image area you are currently viewing and calculates the intensity to find a better minimum and maximum contrast.

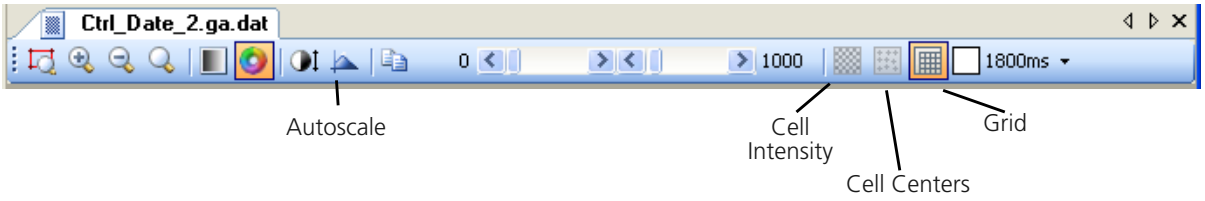
To use the Autoscale function:

- Click the **Autoscale** button.
  The contrast and brightness are automatically adjusted.

Changing the Grid and Intensity Display

These functions only work when DAT files are displayed (Figure A.23).

Figure A.23 Image window tool bar, grid and intensity controls (for DAT files only)



Displaying Cell Intensity Data

If you have a DAT file open with the associated cell intensity data (CEL) file available, you can view the intensity data in the DAT file Image window.

To display or hide the cell intensity data:

- Click the **Cell Intensity** button .

  The cell intensity data for the array is displayed.

Displaying Different Exposures (GeneAtlas DAT Files Only)

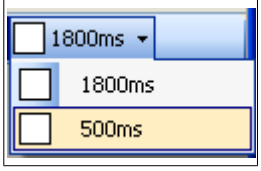
Each GeneAtlas array is imaged twice, with different exposure times. The image data from both exposures are in the GeneAtlas DAT file. You can switch between the different exposures for checking the gridding, etc., by using the Exposure button.

The Exposure button displays the currently displayed exposure time.

To switch between views of the different exposures:

- Click the Exposure button (Figure A.24).

Figure A.24 Exposure Button



The other DAT Exposure is displayed.

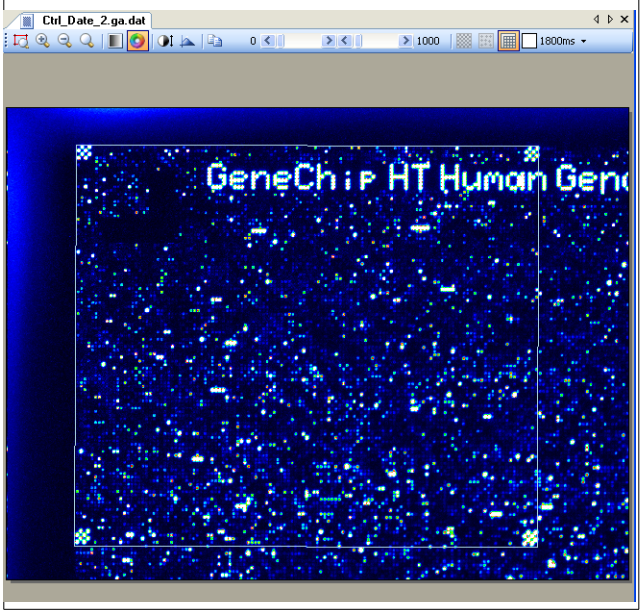
Displaying the Grid

You can display or hide the grid using the Show Grid Corners button.

To display or hide the grid:

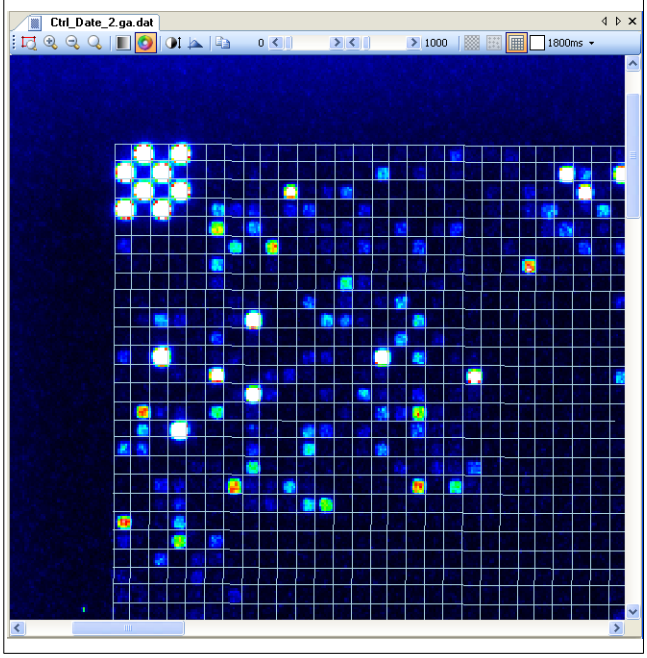
- Click the Show Grid Corners button .

Figure A.25 Outside edges of grid displayed



if you zoom in, the individual grid cells are displayed (Figure A.26).

Figure A.26 Individual grid cells displayed



See *Checking the Grid Alignment* on page 103 of this manual for more information about manual gridding.

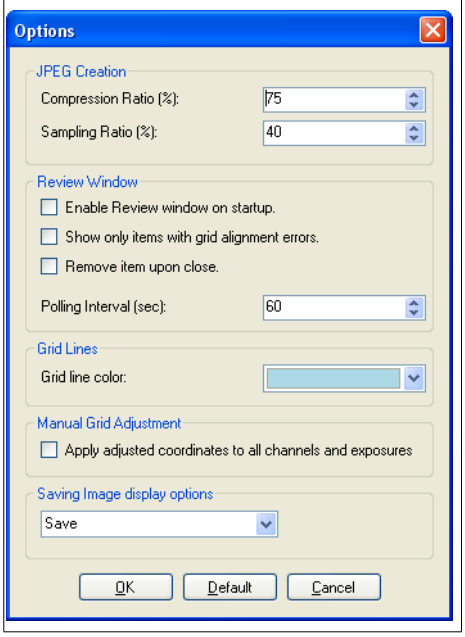
Changing Settings for the Grid Display

To change settings for the grid display:

1. From the **View** menu, select **Options...**

The Options dialog box opens (Figure A.27).

Figure A.27 Options dialog box.



2. Select a new color for the grids from the Grid line color drop-down box.
3. Click **OK** to close the dialog box and enable the changes.

Learning about the Image File

The Properties box displays information about the image file displayed in the window. The information can be displayed in alphabetical order, or ordered by different categories, depending upon the type of file displayed:

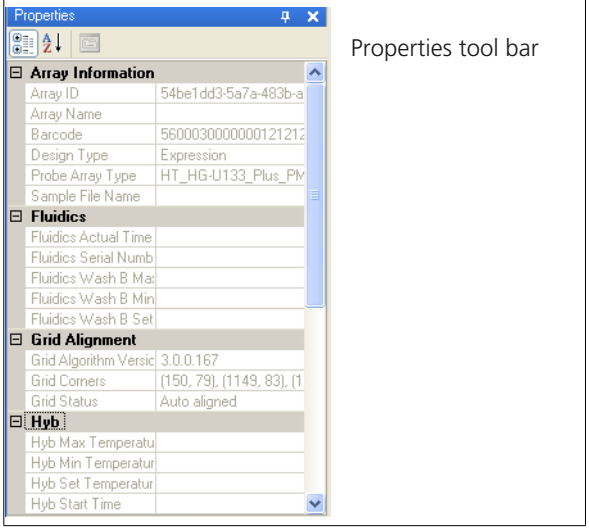
For a DAT file:

- Array Information
- Fluidics
- Grid Alignment
- Hyb
- Image
- Imager

For a CEL file:

- Array Information
- Cel
- Fluidics
- Imager

Figure A.28 Properties box



To expand or collapse a component:

- Click on the +/- button to the left of the component.

To sort the data in a different way:

- Click the **Category Sort** or the **Alphabetical Sort** button.

The Grid information category displays information about the grids:

- Grids (when available): Displays the pixel coordinates for the corners of each grid.

Checking the Grid Alignment

This chapter describes the use of the GeneAtlas Viewer for aligning failed grids:

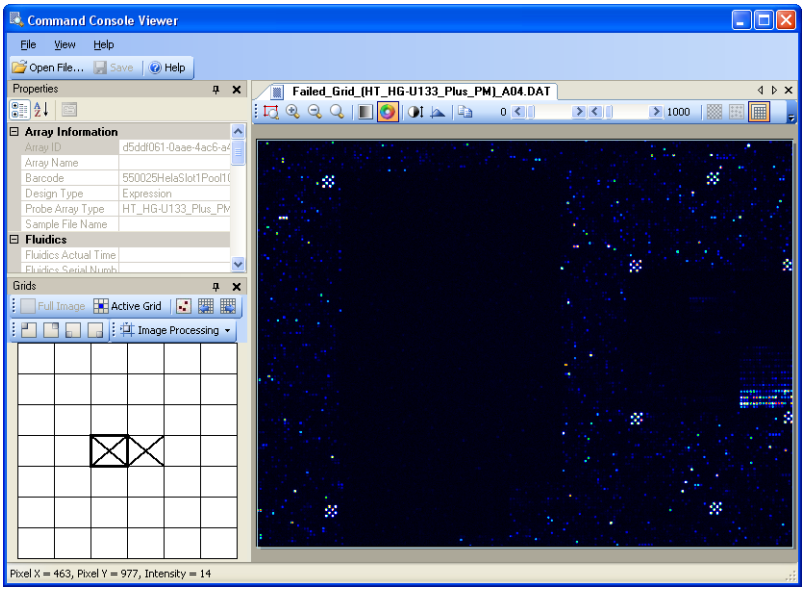
- *Aligning Grids* on page 103
- *Regenerating Intensity Values* on page 110

For general information about grid alignment, see *Array and Grid Types* on page 90.

Aligning Grids

Sometimes one or more grids may require alignment. The failed grids are marked with an X in the Grids box (Figure A.29).

Figure A.29 Array with misaligned grids



If the grid alignment fails you can:

- Run the grid alignment algorithm (see below).
- Perform a manual alignment on the failed grids (see page 105).

You use the same controls and steps to align subgrids for GeneAtlas arrays as you do for cartridge arrays, with the following exceptions:

- You may have to check the grid alignment for both exposures (see *Displaying Different Exposures (GeneAtlas DAT Files Only)* on page 100).
- You can select an option to apply adjusted coordinates to all channels and exposures (see *Changing the Manual Grid Adjustment Setting* on page 109

Running the Grid Alignment Algorithm

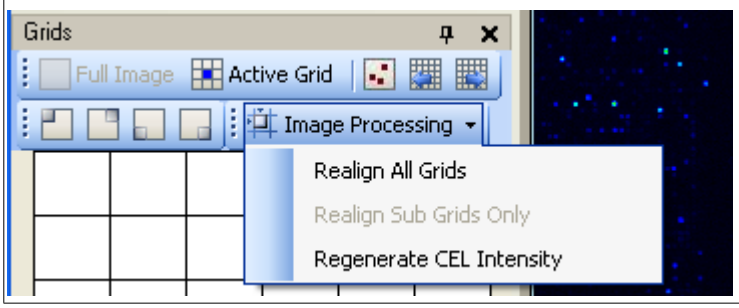
You can run the grid alignment algorithm if some of the grids are misaligned or if you have manually aligned the main grid.

To run the alignment algorithm again on an array that uses sub-grids:

1. In the Grids toolbar, click on the **Image Processing** button 

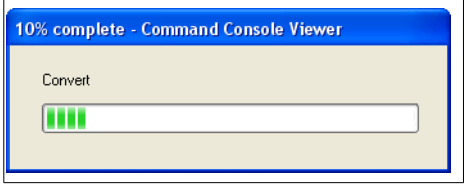
and select **Realign All Grids** from the menu (Figure A.30).

Figure A.30 Grids tool bar for array with grids



Progress bars display the progress of the alignment and cell generation (Figure A.31).

Figure A.31 Progress bars

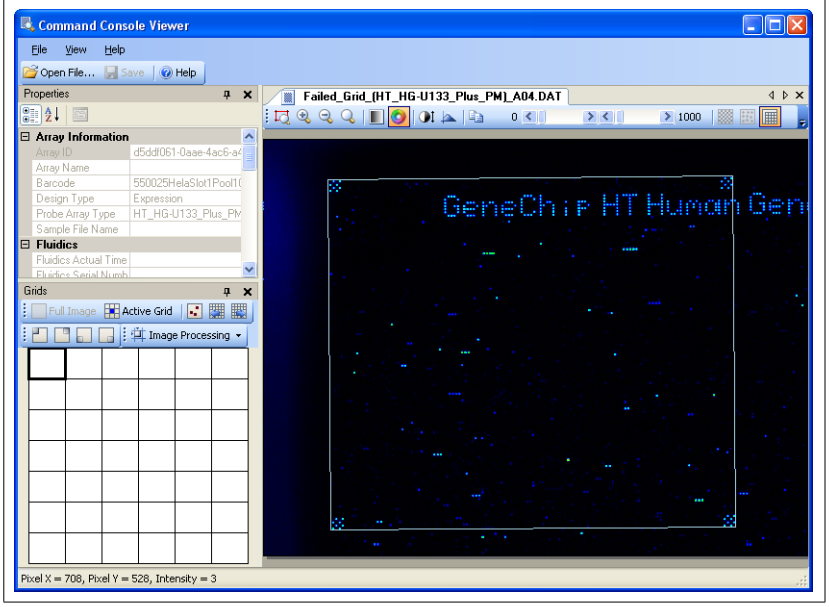


When the process is finished, new gridding information and a new CEL file are generated. A notice of completion appears (Figure A.32).

Figure A.32 Notice

The original DAT, CEL, and JPG files are replaced with new, correctly gridded ones (Figure A.33).

Figure A.33 DAT file after gridding fixed



NOTE: This will align all the subgrids on the array.

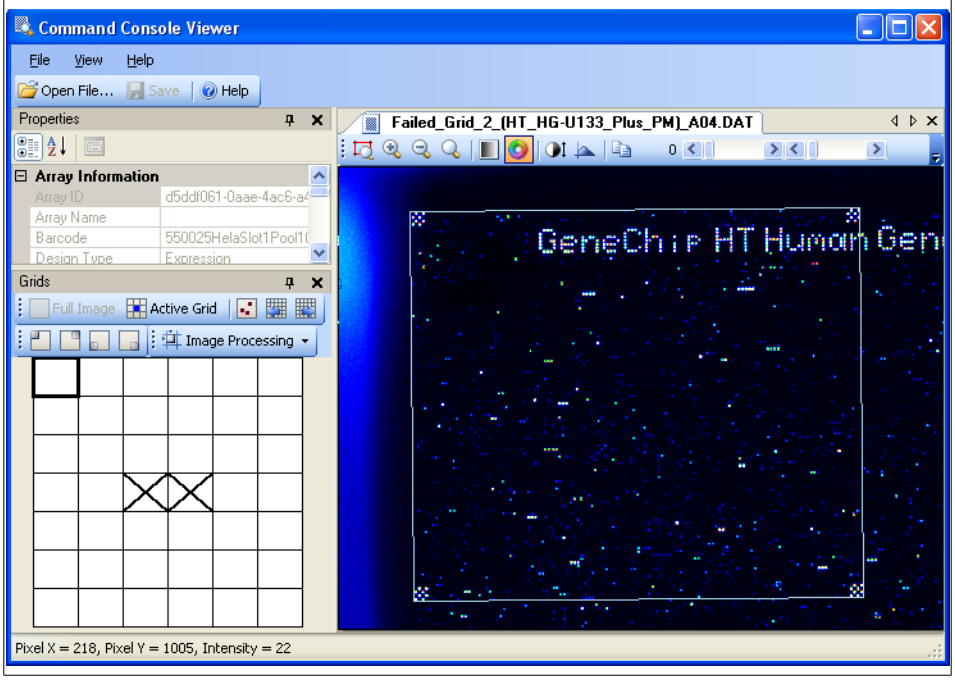
Manually Aligning the Subgrids

If the algorithm alignment fails, an error message appears (Figure A.34).

Figure A.34 Notice of grid misalignment

If you see the error message, you can manually adjust the grid by using the following procedure. The boundaries of a grid are indicated by the alignment patterns at the four corners of the grid.

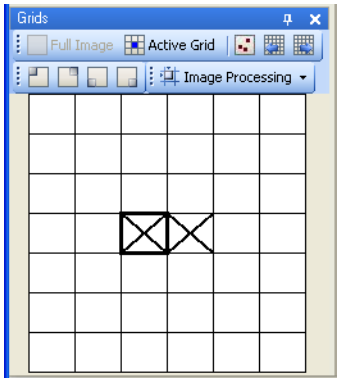
Figure A.35 Grid borders



Navigating from Grid to Grid

The failed grids are marked with an X in the Grids box (Figure A.36).

Figure A.36 Misaligned grids in the Grids box



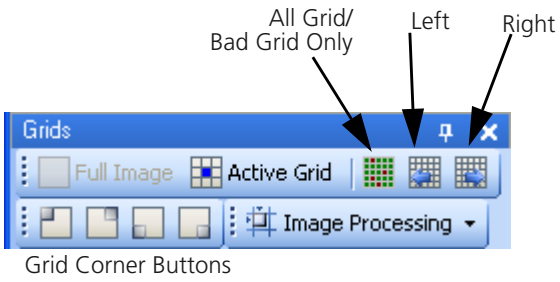
Misaligned grids are marked with an X

Highlighting

- Selected grids are highlighted in black
- Selected misaligned grids are highlighted in yellow
- Modified grids are highlighted in green.

You can step through grids using the right and left buttons (Figure A.37).

Figure A.37 Grid box toolbar, grid step buttons



To step through all grids:

- Click the left  and right  buttons to step through the grids.

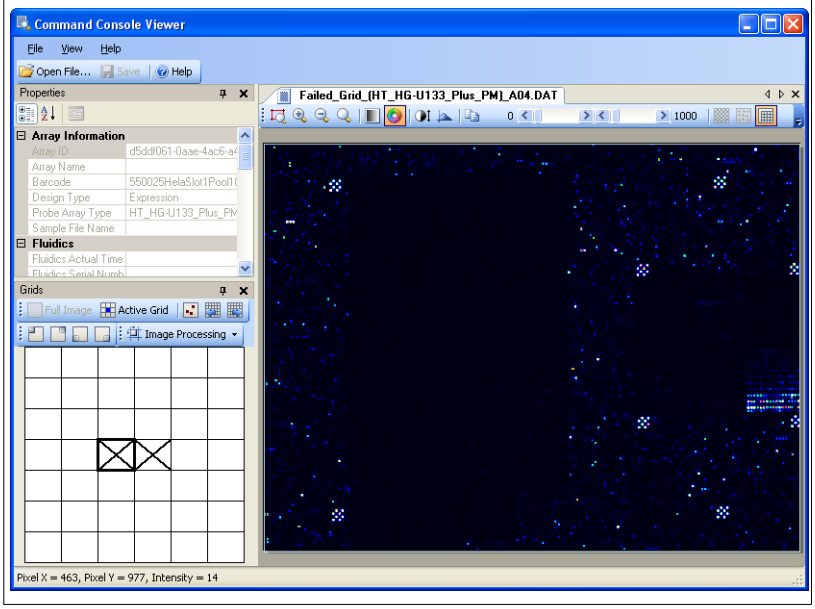
To step only through the misaligned grids

1. Toggle the Step button to the misaligned position.
2. Click the left  and right  buttons to step through the grids.

To manually align a failed grid:

1. Click on the grid you wish to align in the Grid box.
2. A zoomed-in view of the grid appears in the Image window (Figure A.38).

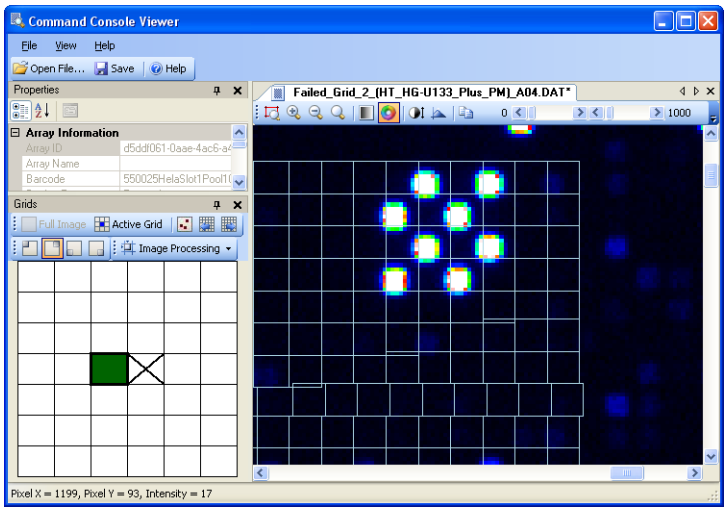
Figure A.38 Selected grid



3. Align the grid at each corner:

   A. Click the **Go To Corner** button for the corner you wish to align. A zoomed-in view of the corner of the grid appears (Figure A.39).

Figure A.39 The upper right grid 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 outside corner of the small checkerboard pattern (Figure A.40).

Figure A.40 Dragging the grid boundary

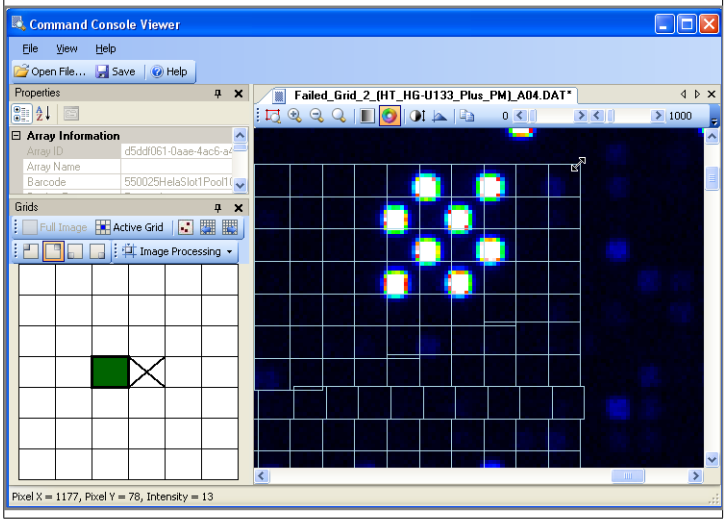
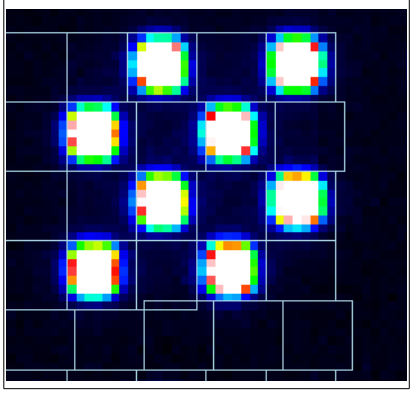


Figure A.41 Correct alignment of corner grid



D. Repeat steps A through C for the other corners of the grid.

4. Continue manually aligning all misaligned grids.
5. After you align the grid, click the **Save** button 

or select **File** → **Save** from the menu bar. The DAT file is saved and new CEL file data is generated.

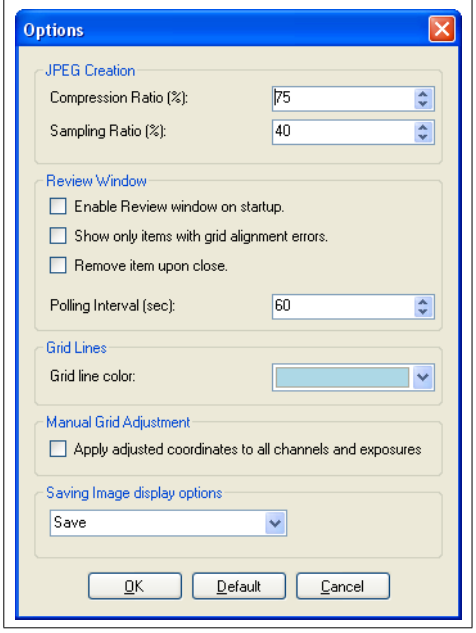
Changing the Manual Grid Adjustment Setting

You can apply the coordinate adjustments made to one exposure time or channel to the other exposure time or channel.

To change settings for the manual grid adjustment:

1. From the **View** menu, select **Options...**
   The Options dialog box opens (Figure A.42).

Figure A.42 Options dialog box.



2. Select or deselect the Manual Grid Adjustment checkbox.
3. Click **OK** to close the dialog box and enable the changes.

Regenerating Intensity Values

Cell intensity values are generated automatically after:

- Running any grid alignment algorithm.
- Saving a DAT file after manual gridding.

You can also regenerate the intensity values without performing one of these other steps.

To regenerate the intensity values:

1. In the Grids toolbar, click the **Image Processing** button 

.
2. Select **Regenerate CEL Intensity** from the list.
   New CEL file data is generated.

Exporting Images in Other Formats

You have two options for exporting a copy of the image:

- *Copying Images to the Computer Clipboard*
- *Creating a JPG File* on page 110

Copying Images to the Computer Clipboard

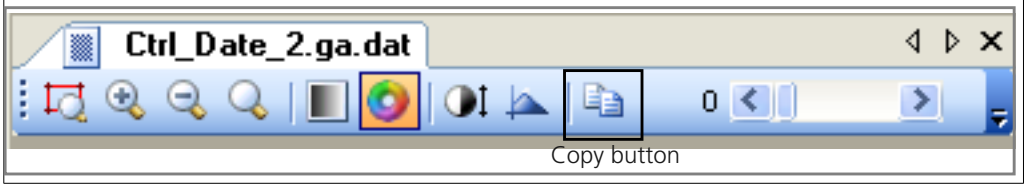
To copy an image to the computer clipboard:

1. Display the image you wish to copy in the Image window.

> **TIP: You can zoom in on a specific region of the image if you desire before copying.**

2. Click the Copy button in the image window toolbar (Figure A.43); or
   Press CTRL-C.

Figure A.43 Copy button



The image in the Image window is copied to the clipboard.
You can then paste the image into a graphics program such as Paint and save it as a graphics file.

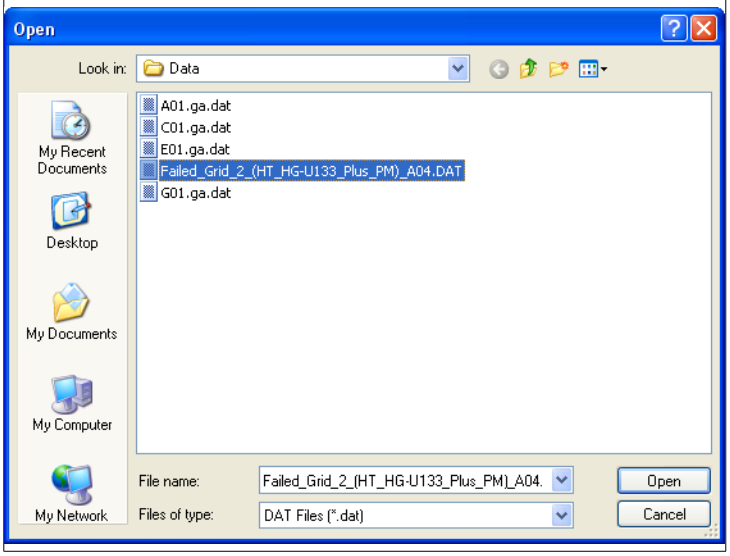
Creating a JPG File

You can create a JPG copy of a DAT file for archive purposes.

To create a JPG copy of a DAT file:

1. From the File menu, select **Create JPG from DAT...**.
   The Open dialog box opens (Figure A.44).

Figure A.44 Open dialog box for JPG creation



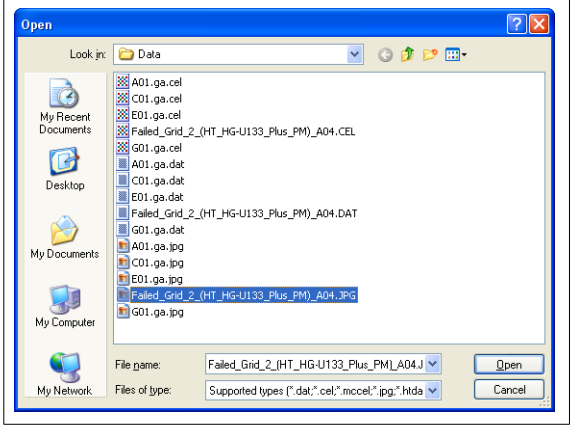
2. Select the DAT file you wish to copy.
3. Click **Open**.
   The JPG file is created.

Viewing the JPG File

To open the JPG file in the GeneAtlas Viewer:

1. Click **File** → **Open File** from the main menu; or
   Click the **Open File** button in the GeneAtlas Viewer tool bar.
   The Open dialog box opens (Figure A.45).

Figure A.45 Open dialog box



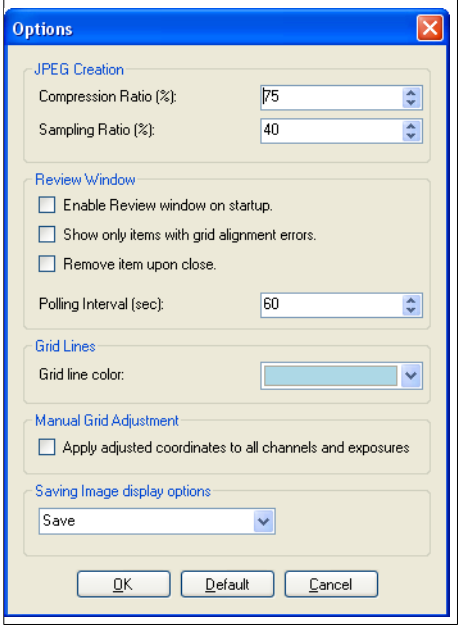
2. If necessary, use the dialog box tool bar to navigate to the directory with the file.
3. Select the file you wish to view.
4. Click **Open**.
   The selected image file is displayed in the GeneAtlas Viewer.

Changing Settings for the JPG Conversion

To change settings for the JPG conversion:

1. From the **View** menu, select **Options...**
   The Options dialog box opens (Figure A.46).

Figure A.46 Options dialog box.



2. Change the values for Compression Ratio and Sampling Ratio.
   Increasing either of these values increases the resolution of the JPG image, but also increases the size of the JPG file.
3. Click **OK** to close the dialog box and enable the changes.

Instrument Care

GeneAtlas® Instrument Care

This chapter provides instructions on caring for and maintaining the instrument, and on troubleshooting if problems arise.

- The GeneAtlas® Instrument instruments should be positioned on a sturdy, level bench away from extremes in temperature and away from moving air.

> **IMPORTANT: Before performing maintenance, turn off power to the station to avoid injury in case of a pump or electrical malfunction.**

Cleaning and Maintenance

The GeneAtlas® instruments require little in the way of customer maintenance. The instruments 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.

- Use soft cloth to clean the surface of the instruments. Do not use liquid cleaner.
- Do not power on and off the instruments frequently. The power off time should be longer than 30 seconds.
- Do not place the instruments on a bench that is not level.
- Do not shake the instruments while moving them from one location to another.

Monthly

Wipe down the outer surface of the instrument with a dry cloth.

Maintenance

Occasionally, you must replace the rear fuses and clean the fan window. The procedures are simple (see and).

Replacing the Fuses

Fuses may be blown because the public power supply network sometimes has problem. The GeneAtlas Imaging Station contains two fuses, the rating of which is 5mm (diameter)×20mm (height), AC 250V, T2.5A for the Imaging Station and AC 250V, T3.15A fuse for the Fluidic Station. There is a marking beside the fuse box which is located next to the power cord plug. You can open the fuse box, remove the fuse holder to replace the fuses with the same rating.

1. Open the rear fuse box by gently prying open with a standard, flat head screwdriver
2. Using the screwdriver, gently pry out the fuse holder.
3. Replace the fuses.
4. Return the fuse holder to the fuse box.
5. Close the box.

Table B.1 The GeneAtlas Imaging Station Fuse Box

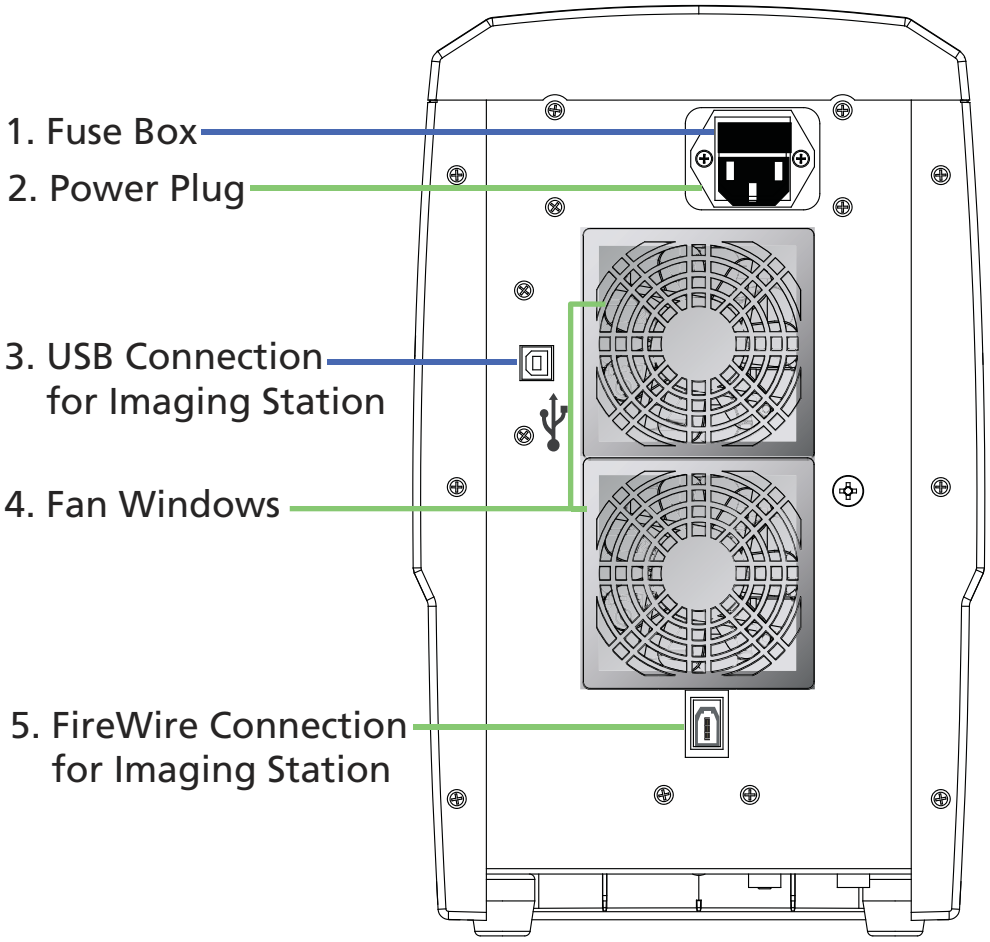
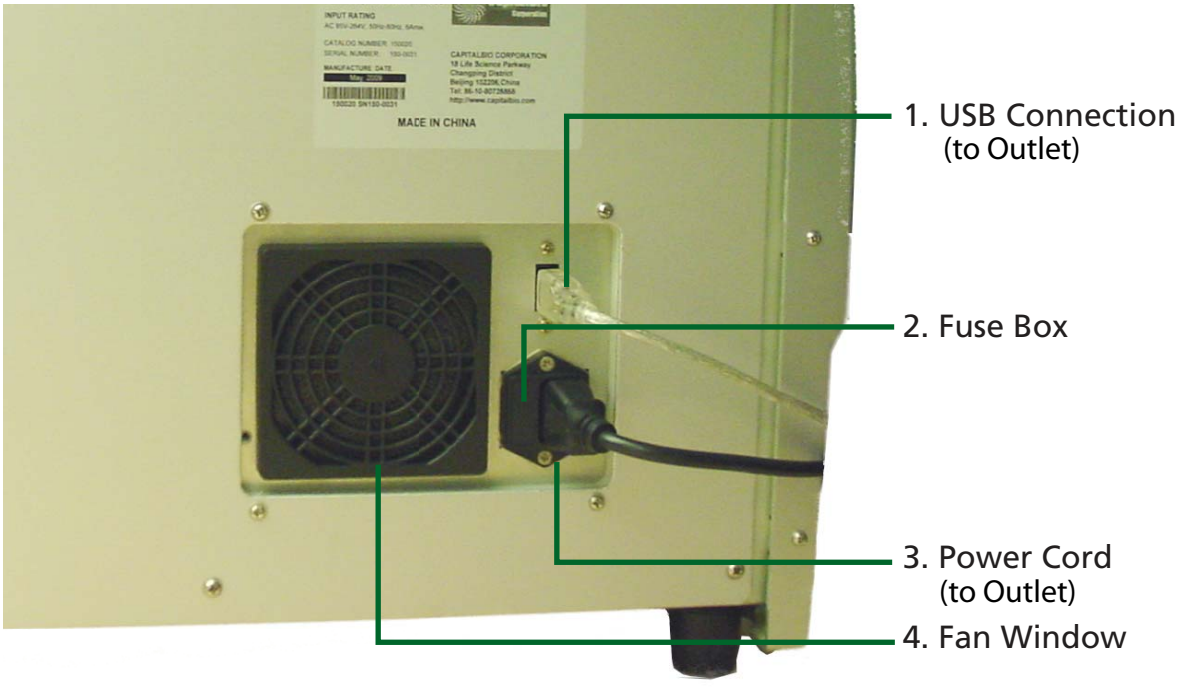


Table B.2 The GeneAtlas® Fluidics Station Rear Fuse Box



Cleaning the Fan Window

The air filter prevents dust or other fine contaminants from entering the Fluidics Station and Imaging Station. You must clean the air filter when you can see a build up of contaminants on the air filter surface.

1. Using a standard flat head screwdriver, gently pry off the fan window.
2. Remove the air filter out from the air filter supporter and
3. Wipe the filter clean using a lint-free cloth.
4. Return the air filter to the supporter
5. Re-install the fan window.

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