Agilent CytoGenomics 2.5
Feature Extraction for CytoGenomics

Quick Start Guide

This Quick Start Guide provides an overview of how to use the Feature Extraction for CytoGenomics software as a stand-alone program, with step-by-step instructions on how to start the program and run an extraction. Although it is not normally necessary to run the Feature Extraction program separately, reading the information in this guide can help you understand how Agilent CytoGenomics extracts data from images during a workflow. For a complete description of the Feature Extraction main window and its components, see the *Feature Extraction for CytoGenomics Users Guide.*

Agilent Technologies

What is Agilent Feature Extraction for CytoGenomics software?

The Feature Extraction for CytoGenomics software is installed as part of the Agilent CytoGenomics software package. Normally, it runs in the background to automatically extract microarray images in Agilent CytoGenomics workflows. It finds and adjusts microarray grids, rejects outlier pixels, accurately calculates feature intensities and ratios, flags outlier features, and calculates statistical confidences. The extracted microarray files are then analyzed by the Agilent CytoGenomics software.

It is not necessary to run the Feature Extraction program separately, except in the following situations:

- Automatic grid placement for a microarray failed and requires manual grid adjustment
- Changes to a protocol are required

Agilent Feature Extraction software extracts data from microarray images produced in three different situations:

- Agilent microarrays scanned on an Agilent scanner
- Agilent microarrays scanned on a supported non-Agilent scanner (i.e. Roche NimbleGen MS 200 scanners, select Innopsys InnoScan scanners, and select Tecan scanners)
- Non-Agilent microarrays scanned on an Agilent scanner (only if the necessary protocol is imported into Feature Extraction for CytoGenomics; download protocols from www.agilent.com/genomics)

Feature Extraction handles each of these images differently. These differences are described throughout the documentation.

If you are a new user

Read "Getting Help" on page 4.

If you intend to analyze...

- Saved Agilent images, read the "Instructional overview" on page 10.
- Saved non-Agilent images, read the "Instructional overview" on page 10.
- Agilent images in real time, read the "Instructional overview" on page 20, and go to "General Instructions" on page 21.

About installed grid templates, protocols, and QC metric sets

When Feature Extraction for CytoGenomics is installed as part of the installation of Agilent CytoGenomics, a standard set of Feature Extraction grid templates, protocols, and QC metric sets are also included in the database. These are displayed when you open Feature Extraction for CytoGenomics.

Grid templates (design files) cannot be imported from within the Feature Extraction for CytoGenomics program; they must be imported as design files from within the Agilent CytoGenomics program. See the CytoGenomics help system for more information.

To update the list of displayed files

Design files and QC metric sets that were created or imported in Agilent CytoGenomics are automatically displayed in Feature Extraction for CytoGenomics when you start the program. Also, protocols and QC Metric Sets that were created or imported from within the Feature Extraction for CytoGenomics program are automatically displayed within the Agilent CytoGenomics program.

To update the list of files displayed in the Feature Extraction for CytoGenomics browsers,

• Right-click in the Grid Template, Protocol, or QC Metric Set Browser, and then select **Refresh**.

Getting Help

To get help for Feature Extraction for CytoGenomics

The following tables list the resources available for getting help. Guides and manuals are opened using Adobe® Reader® software.

Help Resource	Description/Instructions
Feature Extraction Quick Start Guide	This guide, which you are reading, gives you an overview of the Feature Extraction software, including tutorial exercises. To open this guide from within Feature Extraction for CytoGenomics click **Help > Quick Start Guide**.
Feature Extraction User Guide	A comprehensive guide for using the Feature Extraction software, that includes the following chapters:
	• Chapter 1– Working with Feature Extraction • Chapter 2– Extracting Microarrays Automatically • Chapter 3– Creating Grid Files and Templates • Chapter 4– Changing Protocol Settings • Chapter 5– Changing Image Displays To open this guide from within Feature Extraction, click **Help > User Guide**.
Feature Extraction Reference Guide	A reference guide that contains information about protocols, QC reports, results, and algorithms, that includes the following chapters:
	• Chapter 1 – Default Protocol Settings • Chapter 2 – QC Report Results • Chapter 3 – Text File Parameters and Results • Chapter 4 – MAGE-ML Results • Chapter 5 – How Algorithms Calculate Results To open this guide from within Feature Extraction, click **Help > Reference Guide**.
Technical Support	Opens a link to the Agilent Technical Support Web site where you can find literature and manuals, view Frequently Asked Questions, and download updates and revision information. There is also a **Contact Us** link where you can locate the technical support phone number or send an e-mail to technical support for your country.

Help Resource	Description/Instructions
Feature Extraction Training	Opens a link to the Feature Extraction Tutorials Web Site, where you can view a number of self-paced instructional tutorials.
	To open this Web site from within Feature Extraction, click **Help > Feature Extraction Training**.
Feature Extraction on the Web	Opens a menu that provides links to the following:
	• **Array annotation** – Find available design files by barcode
	• **Feature Extraction Software** – Feature Extraction software downloads and information
	• **Feature Extraction Protocols** – Download current and previous versions of Feature Extraction protocols
Agilent License	Opens the Agilent software license redemption Web site, where you use your entitlement certificate to redeem your software license.
Memory Status	Opens an information window that shows how much computer memory is currently available and how much memory is in use.
About	Opens an information window that shows your Feature Extraction version, license, and database information. This window also displays your computer Host ID, which you need in order to redeem your license.

To learn about Agilent products and services

To view information about the Genomics products and services that are available from Agilent, go to www.genomics.agilent.com. To view information about the Life Sciences and Chemical Analysis products and services that are available from Agilent, go to www.chem.agilent.com.

Starting the program

Feature Extraction for CytoGenomics is installed when you install the Agilent CytoGenomics software. A shortcut is *not* installed for the Feature Extraction for CytoGenomics software.

CAUTION	If you install Agilent Genomic Workbench and Agilent CytoGenomics on the same computer, each program installs its own version of Feature Extraction. Do not confuse Feature Extraction with Feature Extraction for CytoGenomics. A shortcut appears only for Feature Extraction version 10.10 (used with Agilent Genomic Workbench.)

To start Feature Extraction for CytoGenomics:

- From Windows, click **Start > All Programs > Agilent CytoGenomicsEdition 2.5.x.x > Agilent Feature Extraction for CytoGenomics.**

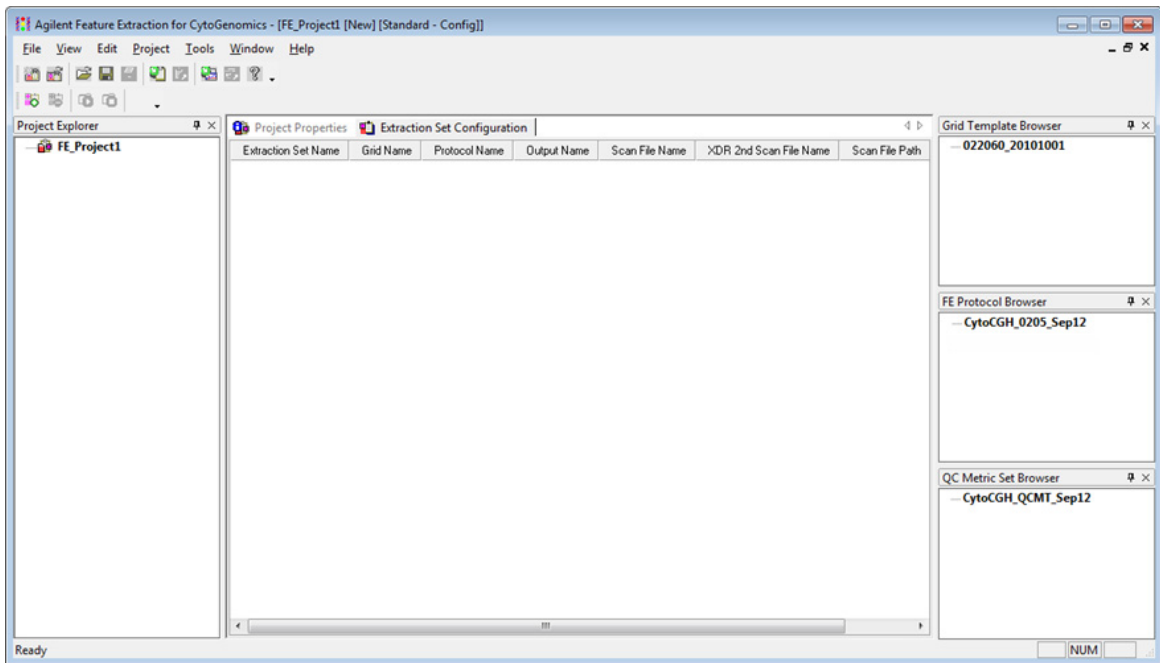Now you see the Feature Extraction for CytoGenomics main window.

Figure 1   Feature Extraction for CytoGenomics main window

To open an image file

1 With the Feature Extraction for CytoGenomics main window open, click **File > Open > Image**, and browse to the folder that contains the image file. Double-click the image file (.tif) that you want to open.

If the Feature Extraction for CytoGenomics program is running, you can also "drag and drop" the image from your folder to the open Feature Extraction window.
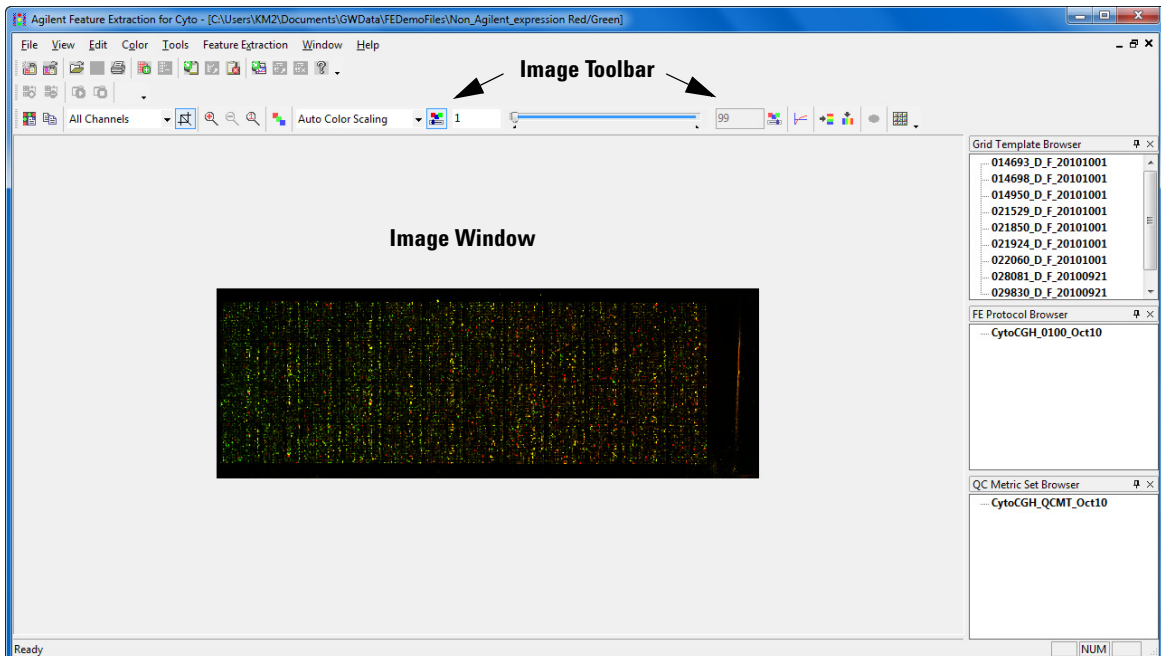

Figure 2 Feature Extraction for CytoGenomics Main Window with open image

NOTE For high resolution images (2 or 3 micron or 20-bit), you see a low-resolution preview image until you crop to roughly 1/10 of the preview image size. Then you see the original high-resolution image. [The Crop Cursor turns red when you are outside the high-resolution (High Fidelity) region.]

To learn more about interactively finding spots and creating grid files for non-Agilent images, see Chapter 3, "Creating Grid Files", in the *Feature Extraction for CytoGenomics User Guide*.
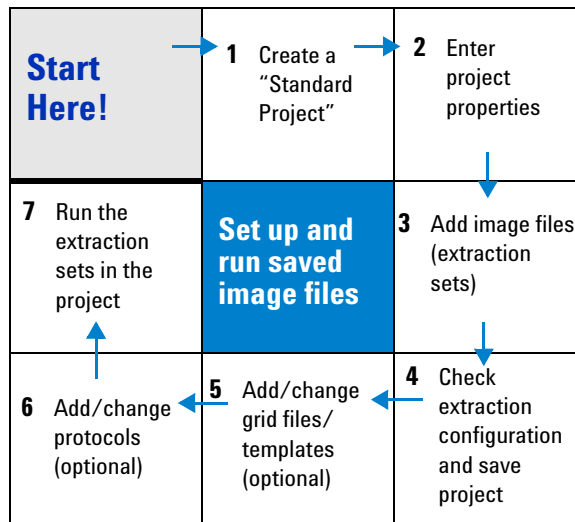
To learn more about changing the image display, see Chapter 5, "Changing Image Displays", in the *Feature Extraction for CytoGenomics User Guide*.

Setting up a Standard Project to extract Agilent and non-Agilent image files

Instructional overview

Follow the road map below and the instructions on the next pages to get started.

See Chapter 2 in the *Feature Extraction for CytoGenomics User Guide* for details on this task.

Start Here!	**1** Create a "Standard Project"	**2** Enter project properties
7 Run the extraction sets in the project	**Set up and run saved image files**	**3** Add image files (extraction sets)
6 Add/change protocols (optional)	**5** Add/change grid files/ templates (optional)	**4** Check extraction configuration and save project

The Agilent Feature Extraction for CytoGenomics program works with Agilent or non-Agilent microarray images produced on Agilent scanners, and Agilent microarray images produced on non-Agilent scanners, using projects, extraction sets, grid templates or files, and protocols. The images are organized into extraction sets that contain the grid and protocol information to run the extraction. The extraction sets, in turn, are organized into projects.
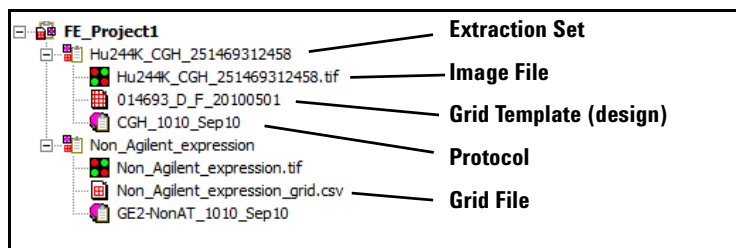
Figure 3   Project Explorer with expanded extraction sets

project   A grouping of information that contains one or more *extraction sets* that define the files to use in the extraction.

extraction set   Each group that contains a microarray image file, grid template (or grid file) and protocol within a project. This term is used both before and after extraction.

grid template   Grid information from Agilent design files or grid files stored in the database. A grid template includes feature annotation and general geometry about the microarray (number of rows, columns, subgrids, feature spacings), which is used to find spots before data analysis takes place. Although not specific to any microarray image, grid template information is usually applied to the image for which the template was designed.

grid file   Grid files are required in order to do feature extraction for non-Agilent images. Rarely, the automatic grid placement of Agilent images using a grid template may produce an incorrect grid, which will also require the use of a user-generated grid file. When using a grid file, extraction uses the nominal and "found" spot locations specific to the image from which the grid file was created. Grid files are created in the Feature Extraction for CytoGenomics program using a microarray image file and a microarray layout file (such as an Agilent Design file or a .GAL [GenePix Array List] file). The grid file contains the locations of the "found" spots if the "Calculate spot size and centroids" option is run before the file is saved. You can create grid templates from grid files, although the templates have only geometry information, no found spots.

protocol   A list of steps and parameter values that define the data analysis algorithms and calculations used for feature extraction of a microarray image or images.

General Instructions

Step 1. Create a Standard Project

- If the Feature Extraction for CytoGenomics program is not already running, start the program according to the instructions given above. See "Starting the program" on page 6

  Feature Extraction automatically creates a standard project (FE_Project1) every time you start the program. The Feature Extraction main window appears with the Project1 work window and an empty Extraction Set Configuration tab window.
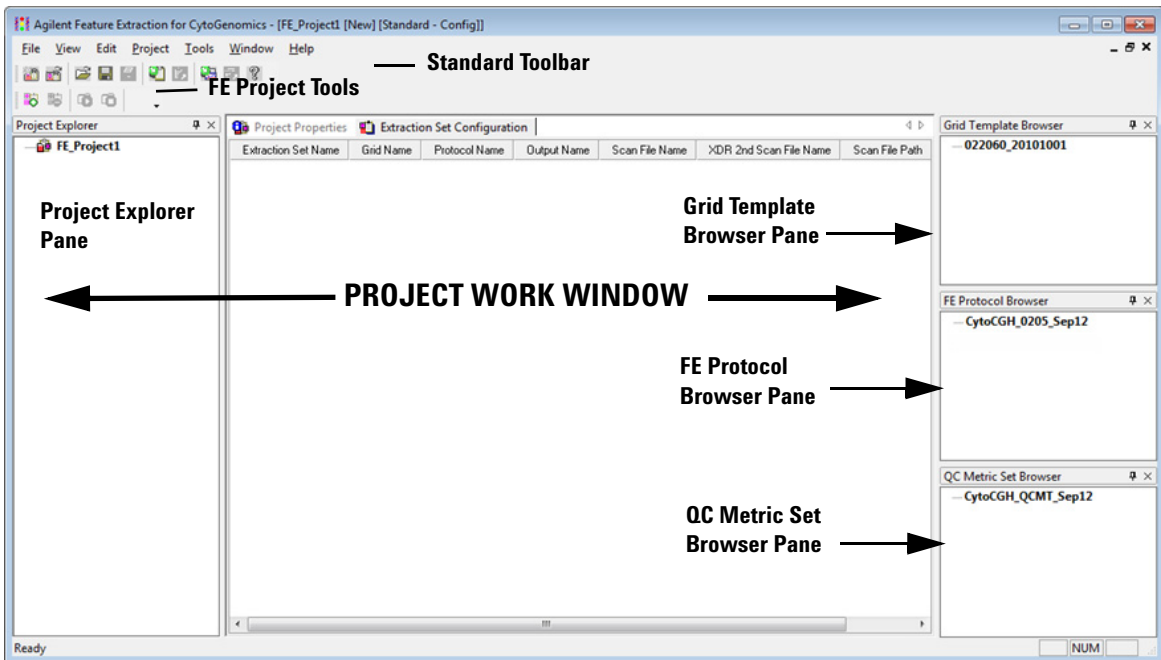

Figure 4   Feature Extraction main window components

You can change where and how the project work window and the browser panes dock when you start the program. To learn more about working with this interface, see the *Feature Extraction for CytoGenomics User Guide.*

Step 2. Enter the project properties

1 Click the **Project Properties** tab to view the default project properties.

2 To change a default setting, select a property and click the down arrow that appears on the right column. Choose from the available options.

Figure 5   Default project properties for a standard project

You can run Feature Extraction for one or more Agilent images generated by the Agilent scanner with these default project properties. You can also set up and run multiple extractions with a combination of Agilent and non-Agilent images.

Step 3. Add image files (extraction sets) to the project

1 In the Project Explorer, right-click **FE_Project1**, and click **Add Extraction**.

2 Select one or more images, and click **Open**.

• To select multiple images at a time, hold down the **Ctrl** key and select the images.

• You can also add one or more image files by dragging and dropping them from the folder to the open project in the Feature Extraction window.

Any extraction set that is missing the required components, such as grid template or protocol, shows a lightning bolt in Project Explorer and a "**?**" in the icon of the missing component. If a grid template is not in the database, you must import it from within the Agilent CytoGenomics program before you run the extraction.

If grid templates are not assigned automatically, or you want to use a different grid template or protocol, you can assign them manually. See Step 4 "Step 4. Assign grid template/grid file to the extraction set (depending on type of images)" on page 14 and Step 5 "Step 5. Assign a protocol to the extraction set (optional)" on page 16.

Step 4. Assign grid template/grid file to the extraction set (depending on type of images)

Agilent images   By default, if the required grid templates are in the database, when you add Agilent tiff images to the project, Feature Extraction will automatically associate the appropriate grid templates to the extraction sets. You can also assign a grid template or grid file to the Agilent image.

To assign a grid template for Agilent images:

1 Expand the extraction set.

2 Select a grid template from the Grid Template Browser, hold down the mouse button and drag it to the second position of the extraction set in Project Explorer.

3 Let go of the mouse button to drop it at this position.

To assign a grid file for Agilent images:

1 Right-click the second position of the expanded extraction set and click **Select Grid File**.

2 Select the grid file, and click **Open**.

The grid file name will appear in the extraction set, along with the grid template on which it is based.

Non-Agilent images   You must assign a grid file to non-Agilent images in the project. The extraction automation feature is not supported for non-Agilent images. Feature Extraction for CytoGenomics will not extract non-Agilent images that are missing grid files or protocols.

To assign a grid file for non-Agilent images:

1 Right-click the second position of the expanded extraction set and click **Select Grid File**.

2 Select the grid file, and click **Open**.

AMADID is the Agilent MicroArray Design IDentifier.

Agilent microarray images scanned with an Agilent scanner   For Agilent microarray images with barcode information included in the image, the program selects a grid template from the database using the AMADID portion of the barcode associated with the image file. If there are grid templates for that AMADID that come from multiple design files, the program selects the grid template from the most recently created Agilent design file. To add a barcode if the image has none, see the *Feature Extraction for CytoGenomics User Guide*.

For this automated assignment to work, the grid template must exist in the database. If it does not, you can interactively associate a grid template or grid file with the extraction set. See for details on how to assign a grid template or grid file.

Agilent uses barcode information for many workflow automation and data tracking steps. Inclusion of the Agilent barcode information in the TIFF file helps ensure that the data is quickly, easily and accurately processed and that informatics programs know how to analyze the results.

Agilent microarray images scanned with a non-Agilent scanner   For Agilent microarray images generated on Roche NimbleGen MS 200 scanners, select Innopsys InnoScan scanners or select Tecan scanners, the program selects a grid template using the same method described above for Agilent microarray images scanned with an Agilent scanner.

Non-Agilent images scanned with an Agilent scanner   Non-Agilent image files from an Agilent scanner do not point to grid information that the Agilent Feature Extraction program can read. Therefore, when you load the non-Agilent image file, you must add a grid file that you have already created.

See Chapter 3 in the *Feature Extraction for CytoGenomics User Guide* to learn how to create grid files.

Step 5. Assign a protocol to the extraction set (optional)

Automatically assigned protocols come from two sources: a default protocol associated with the grid template, or a project level default protocol. You select which of these options has the higher priority. (See the *Feature Extraction for CytoGenomics User Guide* to learn how to associate a protocol with a grid template and where to enter the project level default protocol.) You can also add a protocol to the extraction set manually.

To add a protocol manually:

1 From the Protocol Browser select a protocol, hold down the mouse button and drag it to the third position of the extraction set.

2 Release the mouse button to drop the protocol onto the third position of the set.

Feature Extraction for CytoGenomics can run analyses on Agilent 1-color gene expression (GE1), 2-color gene expression (GE2), CGH, CGH+SNP, ChIP, miRNA, and non-Agilent 2-color GE (GE2-nonAT) microarray images scanned on the Agilent scanner. Feature Extraction for CytoGenomics contains a protocol for extracting CGH and CGH+SNP image types. To run extractions on other Agilent image types (GE1, GE2, ChIP, and miRNA), or on non-Agilent 2-color GE array images, you must first download the required protocol from the Agilent Genomics website, import it into Feature Extraction for CytoGenomics, and then assign it to the appropriate grid template. See the F*eature Extraction for CytoGenomics User Guide* for instructions on importing protocols and associating a protocol with a grid template.

CAUTION These Feature Extraction Protocols were optimized using data from Agilent catalog arrays, which have many replicated probes and validated Negative Control probes. If custom arrays without enough replicated probes are used, or arrays with custom probes designated as Negative Control probes, the default Feature Extraction Protocols may not be optimal. The Feature Extraction Protocol optimization also uses Agilent's default lab protocols; that is, label, hybridization, wash, and scanner procedures.

Step 6. From within the Agilent CytoGenomics program, add design files for the images you intend to extract (optional)

Every extraction set must have an image file, assigned grid template/grid file and protocol. Grid templates and protocols for Agilent images are loaded to the extraction set automatically by the program, if the grid template associated with the loaded image's design file is available in the database. If the design file in the database, it is displayed in the Grid Template Browser. (To update the list, right-click in the Grid Template Browser, and select **Refresh**.) If the design file is not in the database, you must import it manually from within the Agilent CytoGenomics program using the instructions below.

1 Launch Agilent CytoGenomics 2.5.

2 From the Supporting Files tab, click **Import Design**.

3 Select a design file (format *.xml) to import into the CytoGenomics database.

See the CytoGenomics help system for more information on importing design files.

Step 7. Change QC metric set associated with the protocol to monitor QC metrics (optional)

When the microarray processing protocols are similar and monitoring run-to-run consistency is an important goal, Feature Extraction for CytoGenomics can help monitor this consistency with the QC metric sets.

To change the QC metric set associated to the Feature Extraction for CytoGenomics protocol:

1 In the FE Protocol Browser, double-click a protocol to open the FE Protocol Editor.

2 Select **Protocol Properties** to see the protocol properties.

3 Under General, click the cell next to **Metricset**.

4 Click the down arrow, and select the QC metric set appropriate for your microarray application.

If a metric set you want to use is not available in the metric set list, you must first import it to the database.

To import a QC metric set into the database:

1 Select **View > QC Metric Set Browser** from Feature Extraction for CytoGenomics main window, if it is not already visible.

2 The QC Metric Set Browser displays all of the default metric sets and custom metric sets in the database. Custom metric sets are created using the Quality module in the Agilent CytoGenomics program. Right-click in the QC Metric Set Browser and select **Import** to import metric sets that are not shown in the QC Metric Set Browser, or select **Refresh** to update the list.

The default QC metric sets installed with the Feature Extraction software are read-only and cannot be removed from the database. However, you can remove or change a QC metric set associated with the protocol and then save it as a new protocol with a different name.

To remove a QC metric set from the protocol:

• Select **<Set empty>** from the metric set list in the Protocol Properties.

5 Click **Save As** to save the new protocol.

QC metric sets A default CGH QC metric set is loaded into the database when the Feature Extraction for CytoGenomics software was installed.

If you remove a QC metric set, updates of QC metric sets are also available at www.agilent.com/chem/feqcmetrics, if you need to manually download and install them in Feature Extraction for CytoGenomics.

CAUTION   Agilent-supplied QC metric sets are intended to assist users in monitoring microarray processing issues. They were not developed to detect microarray manufacturing issues.

When you create QC metric sets using Agilent CytoGenomics, you can associate them to protocols in Feature Extraction for CytoGenomics. When a project associated with a QC metric set is run, the Feature Extraction for CytoGenomics program summarizes the metric statistics on each microarray's QC report and shows how the results compare to the thresholds (if any) defined in the metric set. It also reports this information in the output files in the statistics table.

Step 8. Save the project

1 Select **File > Save As**.

By default, your projects are saved in one of the following file paths:

- For Windows 7: **C:\ProgramData\Agilent\FeatureExtractionCyto\ FEProjects**
- For Windows XP: **D:\Documents and Settings\All Users\ Application Data\Agilent\FeatureExtractionCyto\FEProjects**

2 To save in a different location, go to or create the folder for your projects.

3 Type a project name, and click **Save**.
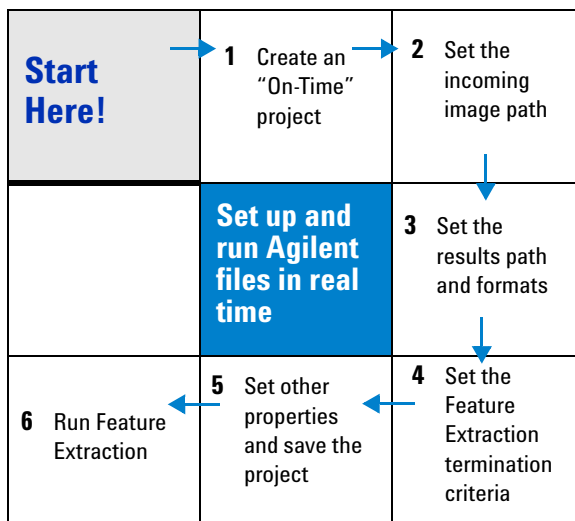
Step 9. Run Feature Extraction

- Click the **Project Run mode On/Off** button on the Feature Extraction Project toolbar , or select **Project > Start Extracting**.

Setting up an On-Time project to extract Agilent image files in real time

Instructional overview

An On-Time project lets you set up the Feature Extraction software to automatically extract Agilent microarray image files as they are transferred from the Agilent scanner to a folder of your choosing.

Follow the workflow below and the instructions on the next pages to get started.

Start Here!	**1** Create an "On-Time" project	**2** Set the incoming image path
	Set up and run Agilent files in real time	**3** Set the results path and formats
6 Run Feature Extraction	**5** Set other properties and save the project	**4** Set the Feature Extraction termination criteria

NOTE	On-Time projects work only with Agilent microarrays scanned on the Agilent scanner.

General Instructions

These instructions help you set up the program to run a batch of Agilent microarray images in real time as they arrive from the Agilent scanner to a folder of your choosing.

Step 1. Create an on-time project

- Select **File > New > On-Time Project**.

  Note that you do not add extraction sets to on-time projects.

Figure 6 On-Time Project Properties window

Step 2. Enter the incoming image path

1 Click the cell next to Incoming Image Folder, and select the path for your scanned images.

2 Click **OK**.

The software assigns the appropriate grid template to each of the Agilent microarrays and the appropriate protocol included in the Grid Template Properties sheet.

Step 3. Enter termination criteria

1 Click the cell next to Use Run Duration Time Out, and select **True**.

2 If not expanded, expand the Duration Time folder.

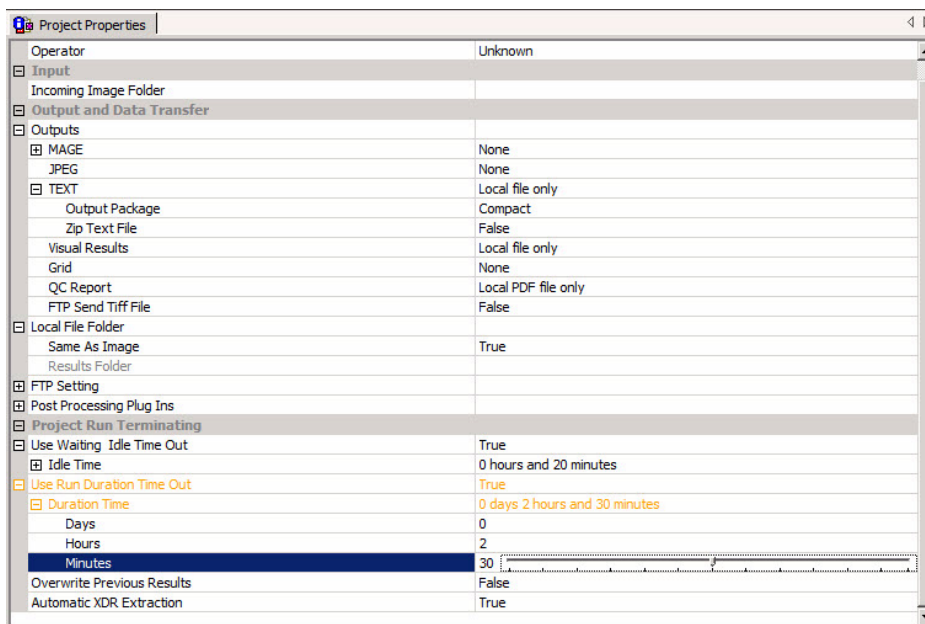**3** Use the sliders to enter 0 for **Days**, 2 for **Hours** and 30 for **Minutes**.

Project Properties		
Operator	Unknown	
Input		
Incoming Image Folder		
Output and Data Transfer		
Outputs		
MAGE	None	
JPEG	None	
TEXT	Local file only	
Output Package	Compact	
Zip Text File	False	
Visual Results	Local file only	
Grid	None	
QC Report	Local PDF file only	
FTP Send Tiff File	False	
Local File Folder		
Same As Image	True	
Results Folder		
FTP Setting		
Post Processing Plug Ins		
Project Run Terminating		
Use Waiting Idle Time Out	True	
Idle Time	0 hours and 20 minutes	
Use Run Duration Time Out	True	
Duration Time	0 days 2 hours and 30 minutes	
Days	0	
Hours	2	
Minutes	30	
Overwrite Previous Results	False	
Automatic XDR Extraction	True	

Figure 7   On-Time Project run terminating

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Step 4. Change QC metric set associated with a protocol to monitor QC metrics

To change the QC metric set associated to the Feature Extraction protocol:

1 In the FE Protocol Browser, double-click a protocol to open the FE Protocol Editor.

2 Select the **Protocol Properties** tab to see the protocol properties.

3 Under General, click the cell next to **Metricset**.

4 Click the down arrow, and select the QC metric set appropriate for your microarray application.

If a metric set you want to use is not available in the metric set list, you must first import it to the database.

To import a QC metric set into the database:

1 Select **View > QC Metric Set Browser** from Feature Extraction main window, if it is not already visible.

2 Right-click in the QC Metric Set Browser and select **Import** to import a metric set.

The default QC metric sets installed with the Feature Extraction software are read-only and can not be removed from the database. But you can remove a QC metric set from the protocol.

To remove a QC metric set associated with the protocol:

• Select **<Set empty>** from the metric set list in the Protocol Properties.

5 Click **Save As** to save the new protocol.

Step 5. Save the project

1 Select **File > Save As**.

By default, your projects are saved in one of the following file paths:.

• For Windows 7: **C:\ProgramData\Agilent\FeatureExtractionCyto\ FEProjects**

• For Windows XP: **D:\Documents and Settings\All Users\ Application Data\Agilent\FeatureExtractionCyto\FEProjects**

2 To save in a different location, go to or create the folder for your projects.

3 Type a project name, and click **Save**.

Step 6. Run an extraction

1 Make sure that the scanner is ready.

2 Click the **Project Run mode On/Off** icon in the Feature Extraction Project Tools, or select **Project > Start Extracting**.

The image files are saved to the Incoming Image Folder as they complete scanning and are then extracted automatically.

You can display the progress of the extractions, the Summary Report, and the QC Report as you did for the Standard Project extractions.

The Summary Report notes how many metrics are within range for each extraction. The QC report for each image contains the values for the metrics and an evaluation of whether they were within or exceeded the threshold limits.

www.agilent.com

In this book

This book contains brief instructions to help you get started with your Feature Extraction for CytoGenomics software. This book shows you:

- How to set up and run batch extractions of existing Agilent and non-Agilent image files

- How to set up and run batch extractions of Agilent image files in real time

Agilent Technologies