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Genomic Workbench 6.5

Feature Extraction 10.10

User Guide

Agilent Technologies

Notices

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In This Guide...

This *User Guide* shows you how to set up and run Feature Extraction automatically for a batch of image files and how to extract image files in real time.

1 Working with Feature Extraction

This chapter introduces the Feature Extraction analysis process and the tasks you can perform with the software to help you with your microarray experiments.

2 Extracting Microarrays Automatically

This chapter provides instructions on how to set up and run Feature Extraction on a batch of existing Agilent and/or non-Agilent image files. It also shows you how to set up and run Feature Extraction on Agilent image files as they are saved from the scanner in real time.

3 Creating Grid Files and Templates

Automatic extraction can take place because grid templates and grid files can be assigned to an image file. This chapter shows you how to create grid files and templates.

4 Changing Protocol Settings

Another reason why automatic extraction can take place is because a set of Feature Extraction parameters can be assigned to an image file as a protocol. This chapter gives instructions on how to change the parameter values for each step of the Feature Extraction process.

5 Changing Image Displays

Learn how to work with image displays in this chapter. This chapter also includes instructions on working with the frames and panes of the interface display.

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Apache acknowledgment

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Non-Agilent microarray image courtesy of Dr. Roger Wagner, Division of Cardiovascular Medicine, Stanford University School of Medicine

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LibTiff acknowledgement

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Content

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1
Working with Feature Extraction

This chapter gives you an introduction to the process of Feature Extraction and lets you know what you can do with the software to optimize the process.

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Feature Extraction process

Feature Extraction consists of two major processes: image analysis to place the grid and locate spots and data analysis to define and measure spot features for gene expression.

Below is a flow chart that shows the process for producing reliable microarray results using Feature Extraction software on Agilent or non-Agilent microarrays scanned on the Agilent Scanner. The data analysis flow starts with removing outlier pixels and ends with transfer of Feature Extraction results into third-party data analysis programs to compare the results of different experiments. This flow chart shows the protocol steps for a Feature Extraction using a CGH protocol.

When you load an Agilent *microarray file* **generated by an Agilent scanner into a** *project,* **the software automatically assigns a** *grid template,* **based on the array AMADID, and a** *protocol.*

The protocol uses the grid template (array design) to place the grid and optimize the grid fit. It automatically locates the spots for Agilent microarrays.

To locate spots on *non-Agilent* **microarray files generated by the Agilent scanner, you must interactively position the grid and save the grid file, which you must load into the project.**

After the software places the grid and finds spots, it uses the remaining Feature Extraction algorithm parameters contained in the Feature Extraction protocol to carry out data analysis.

Flags features and background regions that are population and non-uniformity outliers.

Computes background, bias, and error and performs corrections for these as specified in the protocol settings.

If the image is 2-color, it performs dye normalization and, for gene expression, calculates a *p*-value that is a confidence measure of gene differential expression.

Calculates the log ratios of the dye-normalized green and red channel signals, and calculates final error and significance value.

Protocol Steps

- Place Grid
- Optimize Grid Fit
- Find Spots
- Flag Outliers
- Compute Bkgd, Bias and Error
- Correct Dye Biases
- Compute Ratios
- Calculate Metrics
- Generate Results

Generates the stats table and calculates QC metrics for the QC Report.

Generates for each extracted image the output files (Text, MAGE, QC Report, etc.) that you selected in the Project Properties.

The software automatically sends the Feature Extraction results to certain third-party data analysis programs, such as *Rosetta Resolver® (via FTP)* if you have told it do to so.

You can also run a Feature Extraction *workflow* from within Agilent Genomic Workbench, and automatically analyze the extracted data.

Overview of what you can do with the software

Feature Extraction software window

Automatic batch extraction

You create projects to contain extraction sets. The extraction set includes the Agilent or non-Agilent image file, a grid template or grid file to locate spots and a protocol to specify the values for the parameters to carry out Feature Extraction data analysis.

A list of supported scans and array formats are listed in the following table. Non-Agilent scans are not supported and extraction will not proceed for these scans.

Table 1   Supported scans and array formats

Array	Format	Scan type	Resolution	Support	Project run summary message
Agilent	65um	16 bit	5um	Supported and tested	
Agilent	65um	20 bit	5um	Supported and tested	
Agilent	30um	16 bit	3um	Supported and tested	
Agilent	30um	20 bit	3um	Supported and tested	
Agilent	65um	16 bit	3um	Supported but not tested	Information message
Agilent	65um	16 bit	2um	Supported but not tested	Information message
Agilent	65um	20 bit	3um	Supported but not tested	Information message
Agilent	65um	20 bit	2um	Supported but not tested	Information message
Agilent	30um	16 bit	2um	Supported but not tested	Information message
Agilent	30um	20 bit	2um	Supported but not tested	Information message
Agilent	65um	16 bit	10um	Not Supported	Warning then proceed
Agilent	65um	20 bit	10um	Not Supported	Warning then proceed

Table 1   Supported scans and array formats (continued)

Array	Format	Scan type	Resolution	Support	Project run summary message
Agilent	30um	16 bit	10um	Not Supported	Warning then proceed
Agilent	30um	16 bit	5um	Not Supported	Warning then proceed
Agilent	30um	20 bit	10um	Not Supported	Warning then proceed
Agilent	30um	20 bit	5um	Not Supported	Warning then proceed
non-Agilent	N/A	any	any	Limited Support	Information message

* Limited support means support is provided on a best-effort basis to help you scan microarrays and extract images, however there is no guarantee or commitment. No support means that no support is provided by Agilent Technologies.

When you start an extraction, the software automatically extracts all the sets in the project and produces a project summary report and a QC report for each image. You can also set up a project to automatically extract Agilent microarray images in real time as soon as the files are made available by the Agilent scanner.

Image analysis

Before extraction you can examine the spots on your microarrays visually and compute statistics about the image, using the histogram and line plots provided in the software. You can interactively position grids to find the spots on the microarray and calculate the spot centroids and sizes.

Feature Extraction can handle high-resolution images (2- or 3-micron and 20-bit) from the Agilent G2565CA Scanner. To avoid using large amounts of memory to handle the high-resolution images, Feature Extraction loads a low-resolution preview image for first viewing.

When you create a cropped image to one-tenth of its original size or less, the program loads the original high-resolution image for the cropped image.

Histogram and line plots are always based on the original high-resolution image, not the preview one.

Feature Extraction algorithms for data analysis

For all images, the protocols define features and background regions, remove pixels, and flag features and background regions that may affect the reliability of the results. In addition, protocols set up the extraction to subtract background information from features and make a background adjustment on signals of low intensity.

For GE 2-color, CGH, and ChIP (including DNA Methylation) images, the software will normalize for any differences in dye signal intensity. (SNP probes are not included in these calculations.) After that, the software calculates a reliable log ratio, p-value, and log ratio error for each feature to give you a confidence measure in the measured log ratio.

For microRNA (miRNA) images, Feature Extraction uses a special analysis, described in more detail in Chapter 4.

Extraction results

Feature Extraction offers several types of outputs for each microarray extraction:

- Project Run Summary report
- MAGE (for Rosetta Resolver)
- JPEG (for Rosetta Resolver)
- Text (normal and .zip format)
- Shape
- Grid (used in Feature Extraction only)
- QC Report

You can export the text results containing all the parameters, statistical calculations and feature results into a spreadsheet.

To compare the results of different microarray experiments, you can also import various output types into GeneSpring, Agilent Genomic Workbench software, and third-party analysis programs. The text results can be saved in full, compact, QC, and minimal formats, and MAGE-ML results can be saved in both full and compact formats. For details on what information is included in these files, see the *Feature Extraction 10.10 Reference Guide*.

You can load and display the results to see the outcome of the statistical and flagging algorithms for each feature and background.

What you can do to set up and run automatic batch extraction

Setup

See the Feature Extraction Quick Start Guide for definitions of project, extraction set, grid template and protocol.

You can perform the following tasks when you set up automatic batch extraction:

- Create projects and save them with new names.
- Set up the software to automatically log into the eArray server to download and install the required grid templates with associated dye normalization gene lists (if available), protocols, and associated QC metric sets that are missing in the database.
- Load image files to create extraction sets. For Agilent image files, the software automatically:
  - Loads the required grid template (with associated DyeNorm List, if available) and associated default protocol for each extraction set, if these are available in the database.
  - Check the eArray server for the required grid templates and protocols that are missing from the database.
- Drag and drop scan files onto the project.
- Type or change the protocol or grid template for the project.
- Drag and drop grid templates or protocols onto each extraction set.
- Assign a grid file to an extraction set.
- Choose the type of result file to save.
- Set up to transfer the files via FTP after extraction.
- Delete extraction sets from the project.
- Remove grid templates and protocols from the database.
- Add grid templates, protocols, and QC metric sets to the database.
- Edit protocols and grid template properties.

- Change the order that extraction sets are processed.
- Choose to automatically stop Feature Extraction after a specified time when extraction is in real-time mode (On-Time Project).
- Load 2 micron, 3 micron, 5 micron and 16- or 20-bit image files.

Run extraction

- Start and stop extracting.
- Monitor the extraction progress for each extraction.
- View a Summary Report of the extraction set (or batch of extraction sets).

What you can do with images before extraction

View image and change image display

For details on how to view and change image display, see Chapter 5, "Changing Image Displays".

- Change color and/or scale of the image for easier viewing.
- Select rectangular and elliptical regions of interest.
- Create histograms and line graphs.
- Create images for other programs.
- Print the images.
- Compute statistics about the image
  - Line plots
  - Histogram plots
- Type and save barcode information for Agilent images where the barcode reader has failed.
- Flip and rotate the image from landscape to portrait mode and vice versa, for scans of third-party arrays (cannot do for high-resolution images).

Find grid and spots on the image manually

For details on manual gridding, see Chapter 3, "Creating Grid Files".

For Agilent images, grid templates are applied automatically. You would not normally need to adjust the grid unless an error indicating a gridding failure appears in the Summary Report for an extraction. For non-Agilent images, you must create a grid file before you can extract the image.

- Interactively adjust the grids on the microarray scan.
- Interactively adjust the spots on the microarray (for third-party microarray images only).
- Calculate the spot size and compare the position of the nominal spot centroid (+) laid down by the grid with the found centroid position (X) for the spot (for third-party microarray images only).

- Move the found centroid position (X) to where you want it on the spot (for third-party microarray images only).

  - Feature Extraction uses these manually adjusted centroid positions to define features rather than those calculated by the Place Grid algorithm.

- Find features by typing feature number, gene name, or probe name (for third-party microarray images only). For Agilent arrays, if a shape file is loaded, you can find features by feature number.

- In Grid Mode, select spots to be ignored from analysis (for third-party microarray images only).

What you can do with protocol algorithms

For details on protocols, see Chapter 4, "Changing Protocol Settings" in this User Guide and the Feature Extraction 10.10 Reference Guide.

Protocols contain the values for the parameters used by Feature Extraction algorithms to calculate intermediate and final results. See "Feature Extraction process" on page 18.

What you can do with the protocol steps

- Select the protocol steps that you want to use.
- Change the default settings for each algorithm in the protocols.
- Reload default settings after you have changed them.

What the protocol steps do for you

These protocol algorithms operate in the following order.

Place Grid
- For Agilent microarrays using a grid template, automatically places a grid on the microarray.
- For any microarray loaded with a grid file that you saved during Grid Mode (i.e. interactive gridding), automatically calculates centroid positions. Any spots that were manually adjusted are left unchanged.
- For any microarray, provides two methods of auto-gridding. The default method is to "allow some distortion". This lets the grid have a feature spacing somewhat different from the spacing found in the grid template. The second choice is "place and rotate only". This assumes that the feature spacing from the grid template is accurate and only lets this grid move to the best location.

- For any microarray, user can set the Enable Background Peak Shift flag in order to get better gridding. Some variations in the results are expected with and without use of this flag as the grid positions obtained differ.

- For any microarray, user can select to use regions near to the center of packs for slope and skew calculations. This option is of use in cases where pack edges have dim spots and are failing to grid. When this is selected, the user can select to have the software to perform one extra step of correlation following the projection data analysis to get the origin.

Optimize Grid Fit
- For microarrays with 30- or 65-micron feature size, iteratively adjusts the grid by looking at spots in the corners of the microarray to improve overall image registration.

Find Spots
- Finds the center of each located spot and encircles the pixels belonging to the feature using one of two selected methods: CookieCutter or WholeSpot.

- Calculates statistics for the pixel populations of the features and backgrounds, removing outlier pixels.

- Calculates signal statistics for inlier pixels.

Flag Outliers
- Flags features and background regions that are population and non-uniformity outliers.

Compute Bkgd, Bias and Error
- If you select to subtract the background, subtracts background from the raw signal of each feature using a local or global background method that you select.

- Corrects for spatial gradient across each microarray and any dome effects.

- Adjusts the subtracted signal for inaccuracies in the noise level estimation.

- For all data, also calculates the error based on a selected error model.

- Determines if the feature signal is greater and significantly different from background using the error model calculation.

• Can calculate signals and background for low-intensity features using surrogates.

Correct Dye Biases

• Automatically determines if a dye normalization gene list exists for the grid template.

• Selects to use dye normalization list or generates one internally.

• Selects the features for dye normalization based on a feature selection method of your choosing.

• Performs dye normalization on the normalization feature set with a curve fit of your choosing. (SNP probes are not included in dye normalization calculations.)

Compute Ratios

• Calculates the log ratio of the dye-normalized green and red channel signals.

• Calculates a final error of the log ratio and a significance value (*p*-value) based on an error model that you selected in the Compute background step.

MicroRNA Analysis

• Only available in the miRNA protocol.

• Calculates the TotalGeneSignal for microRNA (miRNA) microarray images only.

This analysis includes all the previous Feature Extraction steps but does not correct for dye biases, or compute ratios.

See Chapter 4, "Changing Protocol Settings" of this guide to learn how to change protocol settings for the miRNA microarray image extraction.

See the *Feature Extraction 10.10 Reference Guide* to learn more about the algorithm in this step.

Calculate Metrics

• Generates the stats table and calculates QC metrics for the QC Report.

You must leave this option turned on to generate a complete stats table and QC report. Some values will be missing if this option is turned off.

See the *Feature Extraction 10.10 Reference Guide* for details on QC metrics.

Generate
Results

- Generates for each extracted image the output files (Text, MAGE, QC Report, etc.) that you selected in the Project Properties.

- Also generates a file for the microRNA image, in addition to the output files (Text, MAGE, QC Report, etc.) that you selected in the Project Properties.

  The file is a GeneView.txt file and contains the SystematicName, the ControlType, the gTotalGeneSignal and the gTotalGeneError for the miRNA microarray.

  See the *Feature Extraction 10.10 Reference Guide* to learn more about this text file.

What you can do with .shp files of extracted images

- Show and hide feature information on the image:
  - Feature number
  - Gene name
  - Raw intensities per color channel
  - Log ratio (rProcessedSignal/gProcessedSignal) for 2-color microarray images
- Show or hide outlier features on the image.
- Search and display one feature at a time using feature number.
- Get reference to annotation results from **Help > Reference Guide**.
- Print the images.
- View the pixels used in the calculation of a feature or set of features.

What you can do with extraction results

- View a QC report for GE 2-color, GE 1-color, and miRNA, each with or without Agilent spike-ins, and for CGH. You can use the QC report to evaluate if the grid was placed correctly (snapshot of four corners of the grid) and to show if the microarray scan data is reliable.

- View all the extraction parameters, statistical calculations and feature results created by the Feature Extraction software to know how reliable each feature result is.

- Export the text file containing the numbers described above to a spreadsheet or a database of your choice.

- Import the extraction results to Agilent Genomic Workbench in order to analyze them using one of the DNA Analytics modules (CGH, ChIP, or CH3) or Quality tools.

NOTE	Extracted data is not automatically sent to Quality tools in Agilent Genomic Workbench when you run an extraction using the Feature Extraction software. However, if you run an extraction using a Workflow in Agilent Genomic Workbench, the data is automatically added to the Quality tools.

- Automatically export MAGE-ML files to Rosetta Resolver (FTP) or manually load the files into a database of your choice.

2
Extracting Microarrays Automatically

You set up to extract a microarray image through the use of *projects*. Projects let you automatically extract one or more image files either as:

- A batch of existing Agilent or non-Agilent files that you add to the project (Standard Project).

- One Agilent image at a time as scanning completes (On-time Project).

This chapter explains how to set up and edit projects, run feature extraction, and view the results.

For information on how to use the Workflow function of Agilent Genomic Workbench to automate Feature Extraction, see the Agilent Genomic Workbench *Workflow User Guide*.

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Setting up eArray login for automatic updates

Feature Extraction can connect to the Agilent eArray server to automatically download, install, and use the required grid templates, dye normalization gene lists, Feature Extraction protocols, and QC metric sets during extraction.

It is highly recommended that you set up an eArray login before you run the extraction project.

1 Double-click the **Feature Extraction** shortcut on your desktop to open the software.

This shortcut was created when you installed the Feature Extraction program.

When you first open the Feature Extraction program, the **eArray Login Setting** window appears on top of the Feature Extraction Standard Project main window. See Figure 1 on page 36. If you do not see the eArray Login Setting window, click **Tools > eArray Settings**.
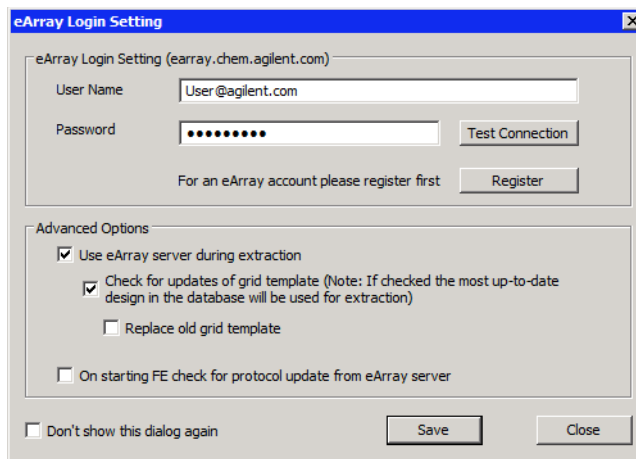
Figure 1   eArray Login Setting dialog box

2 Type your **User Name** and **Password** in the eArray Login Setting window, if you already have an existing eArray account.

Hint: The user name is the e-mail address you provided when you created an eArray account.

If you do not have an eArray account, you must first register for one by clicking the **Register** button. This will take you to the Agilent eArray registration Web site where you can request for a free eArray account. Follow the instructions provided on the eArray Web site to complete the registration.

3 Click **Test Connection**. The message "Login Successful!!!" pops up if the connection to the eArray server is successful. If you get the message "Login denied", you should check that the user name and password you typed are correct.

4 In the Advanced Options, check the appropriate boxes:

- **Use eArray server during extraction**

   Select this box to let Feature Extraction log into eArray server to download, install, and use grid templates (array design files), dye normalization gene lists, Feature Extraction protocols, and QC metric sets during extraction.

- **Check for updates of grid template**

   Select this check box to download updates to existing grid templates already in the Grid Template Browser. Updated grid templates are added to the database, leaving existing grid templates, unless you mark **Replace old grid template**.

   If more than one version of a grid template (design) is in the database, and you want to perform a feature extraction using an older grid template version, clear this check box. Otherwise, Feature Extraction will automatically use the newest version of the grid template (whether or not you have selected to use eArray server during extraction).

- **Replace old grid template**

  When you mark this check box, Feature Extraction will replace old grid templates in the Grid Template Browser with the latest ones from the eArray server.

- **On starting Feature Extraction check for protocol update from eArray server**

  Select this box to have Feature Extraction download updates to existing default protocols in the FE Protocol Browser with the latest one from eArray server every time you start the Feature Extraction software.

5 To save the eArray login settings, click **Save**.

  You will get a message "Setting updated successfully". Click **OK** to close the message.

6 Click **Close** to exit the eArray Login Setting window.

  Now you see the Feature Extraction Standard Project window with FE_Project1 displayed in the Project Explorer as shown in Figure 2 on page 40.

Setting up/editing projects

You set up to extract a microarray image through the use of *projects*. Projects let you automatically extract one or more image files either as:

- A batch of existing Agilent or non-Agilent files that you add to the project (Standard Project).

- One Agilent image at a time as scanning completes (On-time Project).

To set up/edit a Standard Project to run existing image files

You can run Feature Extraction for Agilent or non-Agilent microarrays automatically through the use of standard projects. To each standard project, you add one or more *extraction sets*, which consist of the *image file*, a *grid template or file* and a *protocol*. The protocol contains all the values and settings for the parameters used in each step of the Feature Extraction process.

To learn more about working with individual images and with the Feature Extraction software window, see Chapter 5, "Changing Image Displays".

Figure 2 Feature Extraction Standard Project window

*If you do not see the QC Metric Set Browser, click **View > QC Metric Set Browser**.*

1 Open a Standard Project, if one is not already open.

See "Create a Standard project" on page 42.

2 Change Project Properties.

See "To display project properties for individual projects" on page 45.

3 Add the image files to make up extraction sets.

See "Working with extraction sets" on page 71.

4 View the Extraction Set Configuration tab sheet to make certain all information is correct for each extraction set.

See "To display/edit the extraction Set Configuration table" on page 76.

5 For any extraction set with a non-Agilent microarray image, add an appropriate grid file.

See "To add or change a grid file to an extraction set" on page 84.

6 For any extraction set with a non-Agilent microarray image, add a protocol.

See "To add a protocol to an extraction set" on page 100.

Starting with Feature Extraction 10.5, protocols are linked to QC metric sets. A QC metric set is no longer assigned to the Project Properties tab. Instead, a QC metric set is assigned to a protocol. You can add or change the default QC metric set associated to the protocol.

See "Working with QC metric sets" on page 104.

7 Save the project.

See "To save a project" on page 44.

To set up/edit an On-Time project to run image files in real time

You can run Feature Extraction for Agilent microarrays in real time as image files are saved after scanning on the Agilent Scanner. The differences between a Standard project and an On-Time project are listed below:

• You can set up an On-Time project only for Agilent microarray images. Non-Agilent microarray images are not supported.

• You do not add image files or extraction sets (image files, grid templates, and protocols) to the On-Time project.

• You can type a maximum total processing time and a maximum time between extractions for an On-Time project.

If you want the On-Time project to process microarrays whose grid templates are missing from the database, you must set up the software to use the eArray server during extraction. The required grid templates, protocols, and QC metric sets are then automatically downloaded and installed in the database. See "Setting up eArray login for automatic updates" on page 36.

1 Open an On-Time project.

See "Create an On-Time project" on page 42.

2 Change Project Properties.

See "To select a time to terminate real-time extractions" on page 55.

3 Save the project.

See "To save a project" on page 44.

To create a project

Create a Standard project

To create a new standard project:

- Click **File > New > Standard Project**,
  Or
  Click the **New project file** icon.

When you create the next project after FE_Project1, the name is FE_Project2. The name of every project that you create after that follows in numerical order, whether you save FE_ProjectN or not or close FE_ProjectN or not.

Each time you restart the software, the first new project is FE_Project1, if that is the selection in the Preferences dialog box.

Create an On-Time project

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

Notice that the On-Time Project, Figure 3 does not have a Project Explorer and an Extraction Set Configuration tab.
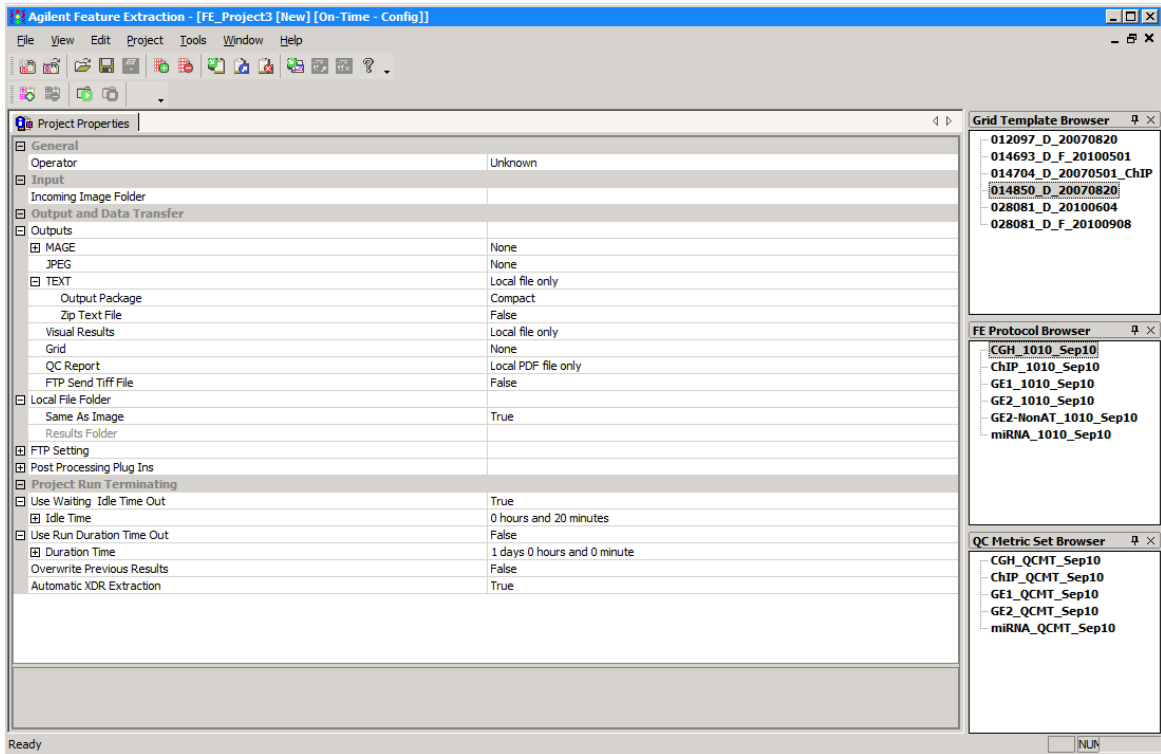
Figure 3   On-Time Project Properties window

To open a project

To open an existing Standard or On-Time project:

1 Click **File > Open > Project**,
Or
Click the **Open project file** icon.

2 Select the project name, and click **Open**.

To save a project

To save a Standard or On-Time project to a new name:

1 Click **File > Save As**.

The default name is FE_ProjectN, where N is the number of the currently loaded project.

2 Type the new name, then click **Save**.

If you are saving the project for the first time, you can click the Save icon and type any name you choose. The default name that appears is FE_ProjectN, where N is the number of the currently loaded project.

The default directory for projects is

C:\Program Files\Agilent\Agilent Genomic Workbench Standard Edition <version>\FeatureExtraction\FEProjects

To save a project to its current name:

• Click the **Save** icon.

If the current project has never been saved, the Save As dialog box appears.

Changing properties for individual projects

If you are changing properties for every project that you run, you probably should change the default properties in the Preferences dialog box. See "Changing default project properties in Preferences" on page 57.

When you create a new project or open an existing project in the Feature Extraction software, you see a Project Properties tab. The default values found on this tab were set in the Preferences dialog box. For each project that you open, you can change these default properties.

To display project properties for individual projects

Project Properties tab for Standard projects

• In the main window for Project setup, click the **Project Properties** tab.

The Project Properties tab for Standard projects with specified extraction sets looks like Figure 4 below.

Figure 4   Standard Project Properties tab, expanded

Project Properties tab for On-Time projects

When you create a new On-Time project or open an existing one, you see the Project Properties tab.

The Project Properties tab for On-Time projects looks like Figure 5 below.

Figure 5   On-Time Project Properties tab, expanded

The parameters and settings in this tab are the same as those in a standard project with these differences:

- No capacity to select an available grid file.

- No setting for default protocol.

- No capacity to select an external DyeNorm list file.

- Capacity to set up a folder for images coming in from the scanner.

  See "To select the incoming image folder for On-Time projects" on page 55.

- Capacity to terminate the run automatically.

  See "To select a time to terminate real-time extractions" on page 55.

- Capacity to automatically extract XDR (extended dynamic range) images.

To change general project property settings

Change to new values

- Double-click the cell to the right of the field name.

  True changes to False and vice versa. For list selections, each double-click brings up a different choice in the list.

  This does not work for check boxes or Browser cells.

- Or, click the arrow to the right of the cell, and select from the list.

Reset changed values to default values

At any time, you can change a modified value back to the original default value.

- Right-click the cell on the right, and click **Reset to Default**.

Change the name of the operator

The only general property that you can change is the name of the Operator.

- Click the cell next to **Operator**, and type the name of the operator.

View the Number of Extraction Sets Included

You cannot change this field. If you add more extraction sets than are visible on screen, you can refer to this field to check the number that you have added.

To select the location for saving and/or sending output files

See the Feature Extraction 10.10 Reference Guide for a listing of all the results that are generated in the output files.

This portion of the Project Properties tab lets you select the ultimate location(s) for the image and result files. See "Select the location for output files" on page 49 and "Select the local file folder for saved results" on page 51.

Select the location for output files

- Click the arrow to the right of the cell next to each of the result or image file types to select one of the following location(s).

Local file only Select to save the files to a local file folder. See "Select the local file folder for saved results" on page 51.

FTP send only Select to send the files to an external location. See "To change the FTP settings to send result files automatically to Rosetta Resolver" on page 52.

Both local file and FTP send Select to save the files both to a local file folder and to an external location.

The following output files are generated and sent to the location(s) you specify:

MAGE result file This file contains results in the MAGE-ML (XML) data transfer format, which lets you transfer the data into Rosetta Resolver and Array Express databases.

You can select to send a **Full Output Package** of MAGE-ML data or a **Compact** (reduced) set of data. And you can choose to send the data in a **Compressed** (ZIP) format, or not.

If MAGE output is selected, you also have the capacity to set and pass data ownership and permissions to Rosetta Resolver.

Expand the MAGE folder, and expand the Rosetta Resolver Data Access Setting folder to view the following fields:

If you do not type names for these settings, no access control information is entered into the MAGE-ML file.

Rosetta Resolver Data Access Settings: User Name   Type the name of the user who will own the data transferred to the Rosetta Resolver database.

Rosetta Resolver Data Access Settings: Control Groups   Type the names of the groups who have permission to use the data transferred to the Rosetta Resolver database. If there is more than one group, separate their names with a colon. For example, if you want the groups Public and Special to see the incoming extraction information, type public:special.

JPEG image file   To create a compressed version of the scan file, you can create a JPEG file. A JPEG file stores a likeness of the original image, which is used for visual reference. This option is for Rosetta Resolver use.

You can control the spatial compression of this JPEG output in the protocol. See "To change the compression factor for the JPEG output" on page 255 of this guide.

Text result file   These results are in a tab-delimited text file that you can import into spreadsheets. The file contains input parameters, statistical calculations and feature results.

Compact mode contains all the columns needed for loading into GeneSpring, DNA Analytics, and Agilent Genomic Workbench.

You can split this file into three files, each containing one of the sections. You can also import this file into GeneSpring data analysis software, either as a **Full** set of data, a **Compact** (reduced), **QC**, or **Minimal**. For a listing of the columns included in these formats, see Chapter 3, "Text File Parameters and Results".

You can select to save the text result file in .zip format. The default for this selection is False.

Visual Results   Visual results from the feature extraction process are saved to a .shp or "shape" file, which only the Feature Extraction program can read.

Grid   This is the grid information saved to grid.csv and (for non-Agilent arrays) feat.csv files. It contains the output of all of the grid placement steps (e.g. the spot centroids and spot sizes).

QC Report   A QC Report is saved to the results directory as a pdf file. The type of QC Report saved is determined by the protocol type for the extracted data: 2-color gene expression (GE), 1-color GE, CGH (full or streamlined), ChIP, or miRNA. For some of the applications (GE1, GE2, and miRNA), spike-in data is also included in the QC Reports.

Send a Tiff file via FTP

Because Tiff files already are located in a local file folder, you can select to send them to an external location via FTP, or not.

- Double-click the cell to the right of FTP Send Tiff File, and select **True** or **False**.

Select the local file folder for saved results

Select to save results in the same local folder as the image   This folder must be the folder that contains the images in the extraction sets.

- Under Local File Folder, double-click the cell next to Same As Image to select **True**, if necessary.

Select to save results in a local results folder   This folder can be any folder that you choose.

1 Double-click the cell next to Same As Image to select **False**.
2 Click the cell next to **Results Folder**.
3 Click the **Browse** button.
4 Select the folder to contain the results.
5 Click **OK**.

To change the FTP settings to send result files automatically to Rosetta Resolver

If you use the same settings repeatedly, the values specific to your installation should be set up in the Preferences dialog box. See "To change the default FTP settings for automatic data transfer" on page 64.

- In the Project Properties tab, expand the FTP Setting folder, and type the values for these FTP settings:

Machine Destination This field is specific to your Rosetta Resolver server name. Please contact your system administrator for the name to use.

Port Type the number of the FTP port on the server. The default is port 21.

User name Type the user name on the server that allows FTP access to the import directory of the server. The default User Name is **resolverftp** for Rosetta Resolver systems.

Password This field already contains the default password for Rosetta Resolver servers. If it has been changed, contact your administrator for the correct password.

File Destination Folder Scan information is sent to this folder. For MAGE results, the default setting is **mage** (/import/mage on the server).

Confirm with your system administrator that the FTP account uses the import directory of the server as the default directory for the transfer.

To set up to automatically assign protocols

If you have configured your eArray settings to use the eArray server (see "Setting up eArray login for automatic updates" on page 36) when you run Feature Extraction, the protocol are assigned based on the application type specified in the grid template. The protocol selected will always

contain the application type name and have the latest date. Application types are: CGH, ChIP, miRNA, and Expression (GE1 and GE2).

Select the highest priority location for the default protocol

The software uses the protocol specified at this parameter step to complete an extraction set if a protocol is available in both the grid template and project. For more information on extraction sets, grid templates and protocols, see "Working with extraction sets" on page 71.

1 Click the arrow to the right of the cell next to Highest Priority Default Protocol.

2 Select **Grid Template Default** or **Project Default**.

Grid Template Default   If selected, the protocol specified in the grid template is assigned to the extraction set. If the grid template contains no protocol, then the software assigns the project default protocol. Use this selection if you add extraction sets to your projects that require different protocols.

Project Default   If selected, the protocol specified as the project default protocol is assigned to *all* extraction sets in the project. If none is specified, then the software assigns the grid template default protocol. Use this selection if you typically add extraction sets that use the same protocol, and this protocol is not the default Agilent protocol assigned to the Grid Template.

Change the project default protocol

1 Click the arrow to the right of the cell next to Project Default Protocol.

2 Select a protocol from the list.

If the protocol you want is not shown on the list, you must import the protocol into the database. See "To import or export a protocol to or from the database" on page 102.

To select to use a grid file

- Double-click on the cell next to **Use Grid file if available** and select **True** or **False** from the list.

True If selected, the software assigns to the extraction set the grid file with the same name as the image file and contained in the image directory.

False If selected, the software does not use any grid files in the image directory even if one that meets the criteria exists.

To select an external DyeNorm list file

To learn how to create an external DyeNorm list from the DyeNormList Editor, see "To create a new internal dye normalization gene list for a grid template" on page 93.

External DyeNorm lists are only used with non-Agilent microarrays. For Agilent microarrays, specify the dye normalization list in the grid template. See "To change the dye normalization gene list in the grid template" on page 92.

If the project calls for a DyeNorm list and the image is a non-Agilent array, use an external DyeNorm list. This list must have the column names in the following order: Probe Name, Gene Name, and Systematic Name. This is the same list order for a DyeNorm list exported by the DyeNormList Editor.

1 Click the cell next to External DyeNorm List File.

2 Click the **Browse** button.

3 Select the .txt file, and click **Open**.

To select to overwrite previous results

- Double-click on the cell next to Overwrite Previous Results and select **True** or **False**.

True If selected, a project extraction can overwrite existing files without warning.

False If False, the project extraction does not start if output files exist with the same name in the same folder.

To select the incoming image folder for On-Time projects

This selection is for On-Time projects only.

1 To assign a folder for incoming images, click the **Browse** button in the cell to the right of Incoming Image Folder.

2 Select a folder for the incoming images, and click **OK**.

To select a time to terminate real-time extractions

This task is for On-Time projects only.

1 Expand Project Run Terminating.

2 Set one or both of the following settings to **True** or **False**.

Use Waiting Idle Time Out If True, you can set an Idle Time in hours and/or minutes. The Idle Time is the time between the arrival of one scan file and the arrival of the next scan file into the real-time image folder.

Use Run Duration Time Out If True, you can set a Duration Time in days, hours and/or minutes. The Duration Time is the time you are willing to wait for the project Feature Extraction to complete.

3 If you set either or both settings to True, expand the Idle Time or Duration Time folder, if not expanded.

4 Type the times.

To turn off the option to automatically extract XDR images

This task is for On-Time projects only.

- Double-click the cell to the right of Automatic XDR Extraction to change the default setting from **True** to **False**.

If the setting is True, the paired XDR images from the Agilent scanner are extracted in a combined fashion and a single output file set covering a broader range of data is generated.

If the setting False, the two images are extracted separately and two output file sets are generated.

Changing default project properties in Preferences

You set the default project properties in the Preferences dialog box. The defaults that you type or change here appear in the Project Properties tab when you create a new project.

To open the Preferences dialog box

If you want to change the default settings and values that appear in the Project Properties sheet for all projects, you open the Preferences dialog box.

1 Click **Tools > Preferences**.

The Preferences dialog box opens to the folder that was last opened.
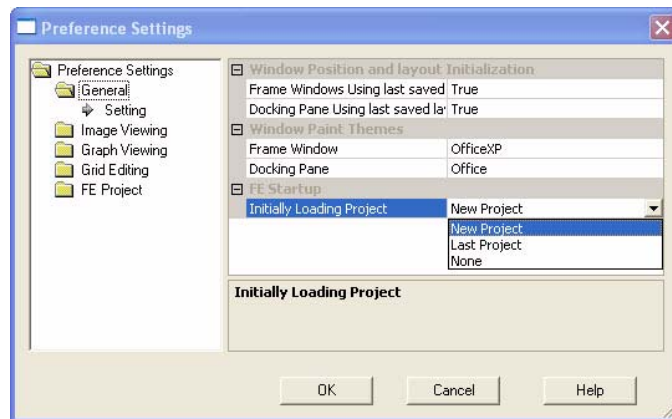
Figure 6   Preference Settings window

To display the General default project properties

1 Open the Preferences dialog box. See "To open the Preferences dialog box" on page 57.

2 Open the **FE Project** folder.

3 Select **General**.
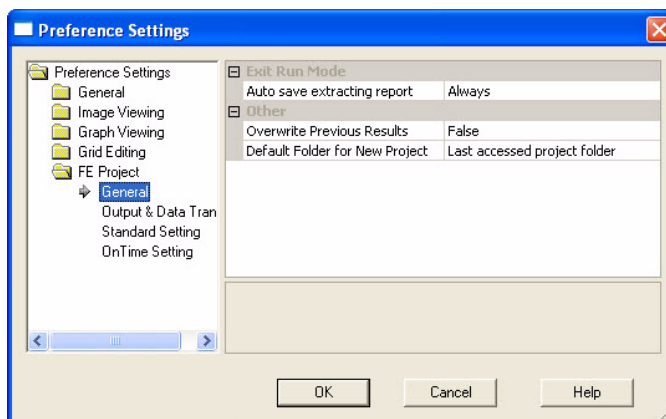
Figure 7   Preference Settings—FE Project General

To select to save the Project Run Summary Report

The Project Run Summary Report contains a history of the extractions run for a specific project. The Project Run Summary Report is initially saved to a temporary .rtf file (YourComputerName_LastBatchReport.rtf) in the directory containing the project files.

This file is overwritten every time an extraction is run on a project and contains only the latest summary report. If you want to save the summary report for each project for later viewing, then you must specify that intention here.

1 Display the General default project properties. See "To display the General default project properties" on page 58.

2 Click the cell next to **Auto save extracting report**.

3 Select from the list.

Always   If selected, the summary report will always be saved after generation as a separate .rtf file in the project file directory. You can view this report for a specific project even after extractions are run on other projects.

Never   If selected, the summary report for that specific project is never saved as a separate .rtf file after generation. You are able to view the report for the project by opening the temporary .rtf file after the project is run. After another project is run, you are not able to access the report for review.

Prompt   If selected, when you exit the project, a prompt appears to ask if you want to save the summary report as a separate file.

To select to overwrite previous results

1 Display the General default project properties. See "To display the General default project properties" on page 58.

2 Double-click on the cell next to Overwrite Previous Results to select **True** or **False**.

True   If selected, a project extraction can overwrite existing files without warning.

False   If False, the project extraction does not start if output files exist with the same name in the same folder.

To select a default folder for a new project

This option defines the initial folder into which you save a new project in the Save As dialog box.

1 Display the General default project properties. See "To display the General default project properties" on page 58.

2 Click on the cell next to Default Folder for New Project.

3 Select one of these options from the list.

Last accessed   Folder you accessed to save the previous project.
project folder

FE Default   Folder set by the application. If you let the installer choose
Project Folder   the default installation folder, the path of this folder is

**C:\Program Files\Agilent\Agilent Genomic Workbench Standard
Edition<version>\FeatureExtraction\FEProjects**

To display the default project properties for Output and Data Transfer

1 Open the Preferences dialog box. See "To open the Preferences dialog box" on page 57.

2 Open the FE Project folder.

3 Select **Output & Data Transfer**.
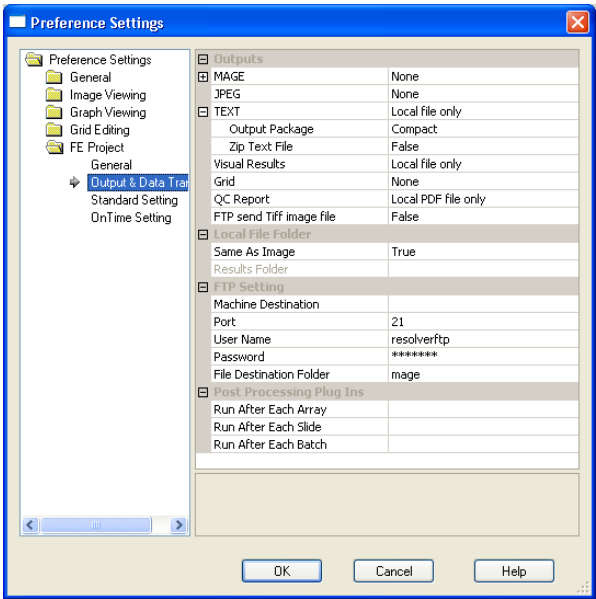
Figure 8   Preference Settings—Output & Data Transfer

To select the default location for saving and/or sending output files

• Display the default project properties. See "To display the default project properties for Output and Data Transfer" on page 60

See the Feature Extraction 10.10 Reference Guide for a listing of all the results that are generated in the output files.

This portion of the Preferences dialog box lets you select the default location(s) for the image and result files. See "Select the default location for output files" on page 61 and "Select the default local file folder for saved results" on page 63.

Select the default location for output files

• Click the arrow to the right of the cell next to each of the result or image file types to select one of the following location(s).

Local file only   Select to save the files to a local file folder. See "Select the default location for output files" on page 61.

FTP send only   Select to send the files to an external location. See "To change the default FTP settings for automatic data transfer" on page 64.

Both local file and FTP send   Select to save the files both to a local file folder and to an external location.

The following output files are generated and sent to the location(s) you specify:

MAGE result file   This file contains results in the MAGE-ML (XML) data transfer format, which lets you transfer the data into Rosetta Resolver and Array Express databases.

You can select to send a **Full Output Package** of MAGE-ML data or a Compact, or reduced, set of data. And you can choose to send the data in a Compressed (ZIP) format, or not.

If MAGE output is selected, you also have the capacity to set and pass data ownership and permissions to Rosetta Resolver.

Expand the MAGE folder, and expand the Rosetta Resolver Data Access Setting folder to view the following fields:

If you do not type names for these settings, no access control information is entered into the MAGE-ML file.

Rosetta Resolver Data Access Settings: User Name   Type the name of the user who will own the data transferred to the Rosetta Resolver database.

Rosetta Resolver Data Access Settings: Control Groups   Type the names of the groups who have permission to use the data transferred to the Rosetta Resolver database. If there is more than one group, separate their names with a colon. For example, if you want the groups Public and Special to see the incoming extraction information, type public:special.

JPEG image file   To create a compressed version of the scan file, you can create a JPEG file. A JPEG file stores a likeness of the original image, which is used for visual reference.

You can control the spatial compression of this JPEG output in the protocol. See "To change the compression factor for the JPEG output" on page 255 of this guide.

Text result file   These results are in a tab-delimited text file that you can import into spreadsheets. The file contains input parameters, statistical calculations and feature results.

A **Compact** data set contains sufficient columns for use by Rosetta Resolver, GeneSpring GX, CGH Analytics or ChIP Analytics software. See the *Feature Extraction 10.10 Reference Guide* for a list of these columns.

You can split this file into three files, each containing one of the sections. You can also import this file into GeneSpring data analysis software, either as a *Full* set of data, *Compact* (reduced), *QC*, or *Minimal* set. For details on what is included in these formats, see Chapter 3, "Text File Parameters and Results".

You can also choose to create a .zip file containing the text results.

Visual Results   Visual results from the feature extraction process are saved to a .shp or "shape" file, which only the Feature Extraction program can read. The compact version of the text file has the columns containing the final processing steps of the data (and other columns needed for import to downstream SW packages). It excludes some columns with intermediate data calculations.

Grid   Choose to save the grid information to grid.csvfiles (for
Agilent and non-Agilent grids) and feat.csv files (for
non-Agilent grids). It contains the output of all of the grid
placement steps (e.g., the spot centroids and spot sizes).

QC Report   A QC Report is saved to the results directory. The type of
QC Report saved is determined by the protocol type for the
extracted data, either 2-color gene expression (GE), 1-color
GE, CGH, ChIP, or miRNA, each one either containing
spike-in data, or not.

Send a Tiff file via FTP

Because Tiff files already are located in a local file folder,
you can select to send them to an external location via FTP,
or not.

• Double-click the cell to the right of FTP Send Tiff File,
and select **True** or **False**.

Select the default local file folder for saved results

Select to save results in the local image folder   This folder must
be the folder that contains the images in the extraction sets.

• Under Local File Folder, double-click the cell next to
Same As Image to select **True**, if necessary.

Select to save results in a local results folder   This folder can be
any folder that you choose.

1 Double-click the cell next to Same As Image and select
False.

2 Click the cell next to Results Folder.

3 Click the **Browse** button.

4 Select the folder to contain the results.

5 Click **OK**.

To change the default FTP settings for automatic data transfer

1 Display the default project properties for Output and Data Transfer. See "To display the default project properties for Output and Data Transfer" on page 60.

2 In the Project Properties tab, expand the FTP Setting folder, and type the values for these FTP settings:

Machine Destination   This field is specific to your Rosetta Resolver server name. Please contact your system administrator for the name to use.

Port   Type the number of the FTP port on the server. The default is port 21.

User name   Type the user name on the server that allows FTP access to the import directory of the server. The default User Name is **resolverftp** for Rosetta Resolver systems.

Password   This field already contains the default password for Rosetta Resolver servers. If it has been changed, contact your administrator for the correct password.

File Destination Folder   Scan information is sent to this folder. For MAGE results, the default setting is **mage** (/import/mage on the server).

Confirm with your system administrator that the FTP account uses the import directory of the server as the default directory for the transfer.

To change default run post-processing plug-ins

1 Display the default project properties for Output and Data Transfer. See "To display the default project properties for Output and Data Transfer" on page 60.

2 In the Project Properties tab, expand the Post Processing Plug Ins folder, and type or select the plug-in you want to run.

The plug-ins are executed independently, so you can run the same or different plug-ins after each event.

Run After Each Array	Select a plug in to run after each array is extracted. All array related arguments are passed to the executable called.
Run After Each Slide	Select a plug in to run after each slide is extracted, where a slide contains 1 or more arrays. Arguments pointing to files relating to the slide are passed to the executable called.
Run After Each Batch	Select a plug in to run after each batch of extractions is completed. Arguments pointing to files relating to the batch are passed to the executable called.

To display the default properties for standard projects

1 Open the Preferences dialog box. See "To open the Preferences dialog box" on page 57.

2 Open the FE Project folder.

3 Click **Standard Setting**.
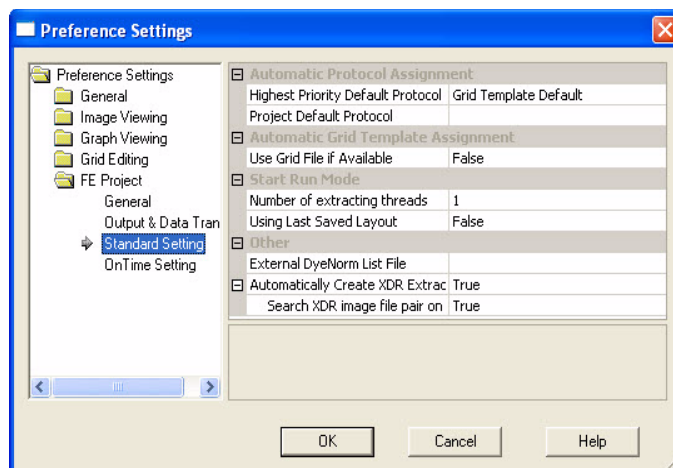
Figure 9   Preference Settings— Standard Project

To change the default protocol and its location

Select the default highest priority location for the default protocol

The software uses the protocol assigned to this location to complete an extraction set if a protocol is available in both the grid template and project. To learn more about extraction sets, grid templates and protocols, see "Working with extraction sets" on page 71.

1 Display the default properties for standard projects. See "To display the default properties for standard projects" on page 65.

2 Click the arrow to the right of the cell next to Highest Priority Default Protocol.

3 Select **Grid Template Default** or **Project Default**.

Grid Template Default If selected, the protocol specified in the grid template is assigned to the extraction set. If the grid template contains no protocol, then the software assigns the project default protocol. Use this selection if you add extraction sets to your projects that require different protocols.

Project Default If selected, the project default protocol is assigned to the extraction set. If none is specified, then the software assigns the grid template default protocol. Use this selection if you typically add extraction sets that use the same protocol, and this protocol is not the default Agilent protocol assigned to the Grid Template.

Change the default protocol for all projects

1 Display the default properties for standard projects. See "To display the default properties for standard projects" on page 65.

2 Click the cell next to Project Default Protocol.

3 Select a protocol from the list.

To select to use a grid file

1 Display the default properties for standard projects. See "To display the default properties for standard projects" on page 65.

2 Double-click on the cell next to Use Grid file if available to select **True** or **False** from the list.

True If selected, the software assigns to the extraction set the grid file with the same name as the image file and contained in the image directory.

False If selected, the software does not use any grid files in the image directory even if one that meets the criteria exists.

To change the Start Run Mode setting

1 Display the default properties for standard projects. See "To display the default properties for standard projects" on page 65.

Number of extracting threads This setting is fixed to one thread. You can no longer change the number of extracting threads.

2 Double-click the cell next to Using Last Saved Layout to select **True** or **False** from the list.

The default setting is False. If True is selected, the software saves the layout changes (i.e. placement of panels and browsers) for the Standard Project and reloads this layout the next time you open Feature Extraction.

To select an external DyeNorm list file

External DyeNorm lists are only used with non-Agilent microarrays. For Agilent microarrays, specify the dye normalization list in the grid template. See "To change the dye normalization gene list in the grid template" on page 92.

To learn how to create an external DyeNorm list from a DyeNormList Editor, see "To create a new internal dye normalization gene list for a grid template" on page 93.

If the project calls for a dye normalization list and the image is from a non-Agilent array, use an external DyeNorm list. This list must have the column names in the following order: Probe Name, Gene Name, and Systematic Name. This is the same list order for a DyeNorm list exported by the DyeNormList Editor.

1 Display the default properties for standard projects. See "To display the default properties for standard projects" on page 65.

2 Click the cell next to External DyeNorm List File.

3 Select the external dye normalization list .txt file, and click **Open**.

To select to create XDR extraction sets automatically

The default for this setting is True. When set to True, the software will automatically create one extraction set for the two images (_H and _L) scanned with extended dynamic range (XDR) turned on in the scanner.

1 Display the default properties for standard projects. See "To display the default properties for standard projects" on page 65.

2 Double-click the cell to the right of Automatically Create XDR Extraction Set to select either **True** or **False**.

If you want to be able to add just one of the two images to the extraction set and have the second image loaded automatically, follow step 3.

3 Double-click the cell to the right of Search XDR image file pair on name matching only to select either **True** or **False**.

True is the default selection. The pair images must be in the same folder for the software to load the second image automatically to the extraction set.

To display the on-time default properties

1 Open the Preferences dialog box. See "To open the Preferences dialog box" on page 57.

2 Open the FE Project folder.

3 Click **OnTime Setting**.
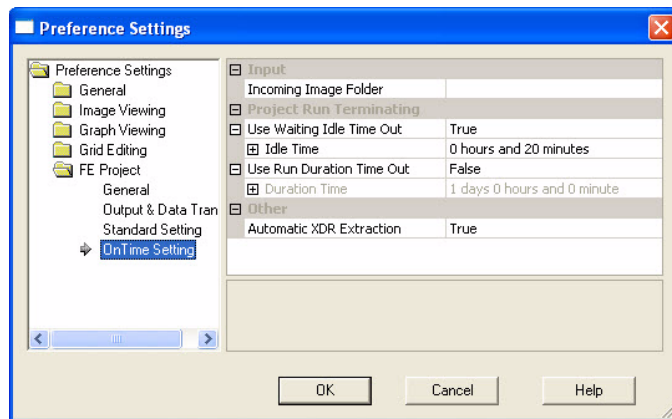
Figure 10   Preference Settings — On-Time Project Properties

To select the default image folder

1 Display the on-time default properties. See "To display the on-time default properties" on page 69.

2 Click on the cell next to Incoming Image Folder.

3 Click **Browse**.

4 Double-click the image folder, and click **OK**.

To select a default time to terminate real-time Feature Extraction

1   Display the on-time default properties. See "To display the on-time default properties" on page 69.

2   Expand Project Run Terminating.

3   Set one or both of the following settings to True or False.

Use Waiting Idle Time Out   If True, you can set an Idle Time in hours and/or minutes. The Idle Time is the time between the arrival of one scan file and the arrival of the next scan file into the real-time image folder.

Use Run Duration Time Out   If True, you can set a Duration Time in days, hours and/or minutes. The Duration Time is the time you are willing to wait for the project Feature Extraction to complete.

4   If you set either or both settings to True, expand the Idle Time or Duration Time folder, if not expanded.

5   Type the times.

To select to automatically extract an XDR image pair

The default setting is True. That is, the software will automatically extract both the High and Low signal images as a single extraction set when the XDR option is turned on in the scanner.

1   Display the on-time default properties. See "To display the on-time default properties" on page 69.

2   Double-click the cell to the right of Automatic Extraction of XDR images to change from **True** to **False**, or vice versa.

Working with extraction sets

What is an extraction set?

To each standard project you add one or more *extraction sets*, which consist of the *image file*, a *grid template or file* and a *protocol*. The protocol contains the values and settings for the parameters used in each step of the Feature Extraction process.
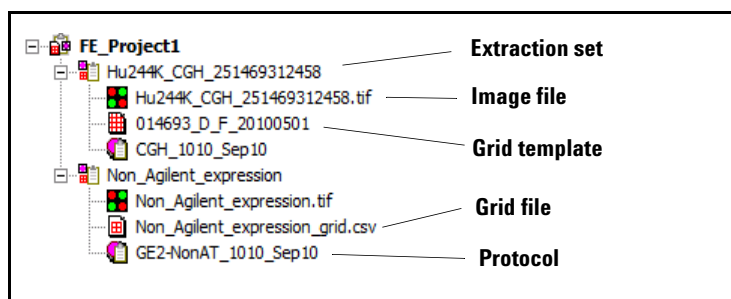
Figure 11   Extraction sets in Project Explorer

extraction set   Each grouping of microarray image (image file), grid template (or grid file) and protocol within a standard project. This term is used both before and after extraction. An XDR extraction includes two image files (_H and _L) and is represented with an icon that marks it as an XDR extraction. See Figure 12.

20-bit single scans replace XDR dual scans. To determine if a scan is 20-bit, click the image file info button, click the Scan Statistics tab, and view the Saturation Value (about 777,000 for 20-bit).
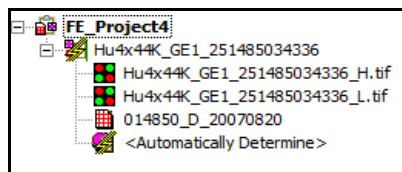
Figure 12   XDR extraction image files marked with _H and _L

grid template
: Grid information from Agilent array design 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 locate spots before data analysis takes place. Although not specific to any image, grid template information is usually applied to the image for which the template was designed.

grid file
: You can also extract using a grid file, which has the nominal and "found" spot locations specific to the image from which the grid file was created. 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 create grid files for non-Agilent images and specific Agilent images that have failed the gridding process.

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.

How does the software create an extraction set?

Step a
: After you add an Agilent image file to the project, the software assigns a grid template from the database by matching the array design file's AMADID to the microarray barcode.

AMADID stands for **A**gilent **M**icro**A**rray **D**esign **ID**entifier. The first 6 digits of the array design file indicate the AMADID. Given a design file 012097_D_20070820, **012097** is AMADID and 20070820 is YYYYMMDD that the design file was generated.

For most Agilent microarray images, the grid template is automatically assigned, if the grid template is in the database. You may need to assign a grid template/grid file manually if, for example, the Agilent microarray image failed auto-gridding on a previous run.

A grid file must be used for a non-Agilent extraction set or if you had to manually grid the microarray.

If the grid template is not in the database, and you have selected to use the eArray server during extraction, the program gets the grid template for the array, based on its barcode, and uses that grid for the extraction. You can also

select to have eArray check for updates to the grid template before extracting. See "Setting up eArray login for automatic updates" on page 36.

Step b   The software also adds a protocol to form an extraction set.

The added protocol is either the default protocol entered with the grid template or specified for the project. You can select which protocol is the first chosen by the software, the protocol in the grid template or the one in the project. If the added grid template or file does not contain a protocol, the software uses the default protocol for the project.

To add extraction sets (image files) to the standard project

You can add an image file or extraction set in one of four ways:

- Drag and drop the image file onto Project Explorer
- Select the extraction set through the FE Project toolbar.
- Select the image file through the Edit menu.
- Select the image file through the Project Explorer shortcut menu

To add image files through Windows Explorer:

1 Open Windows Explorer.

2 Select the file or files that you intend to add.

3 Drag them to the Project Explorer and then drop them.

You now see a list of extraction sets.

4 Expand the extraction sets.

Figure 13   Project window after adding image files (extraction sets)

To add extraction sets through the toolbar:

1 Click the **Add Extraction Set(s)** icon, [icon].

2 Select the image files you want to add, then click **Open**.

You can select one or more images for extraction. Use the **Shift** key to select contiguous files or the **Ctrl** key to select separate files.

To add image files through the Edit menu:

1 Click **Edit > Add Extraction Set(s)**.

2 Select the image files you want to add, then click **Open**.

To add image files through the Project Explorer shortcut menu:

1 Right-click in Project Explorer.

2 Click **Add Extraction**.

3 Select the image files you want to add, then click **Open**.

You can also add extended dynamic range (XDR) extraction sets. These consist of a pair of images, one of high signal and the other of low signal, scanned with the XDR option turned on in the scanner. Feature Extraction extracts both images as a set and creates a single combined set of output files with data covering the combined dynamic range of both scans before it moves on to the next extraction set.

You must make sure that the preferences options for creating and extracting an XDR extraction set are turned on. The default selections are True (ON).

To sort extraction sets

If you want to run extraction sets in an order different from their order in Project Explorer, sort them using these instructions or drag and drop the extraction set in any order in Project Explorer.

1 Click **Edit > Sort Extraction Sets**.

2 Then select from the list — **By Name, By Image, By Grid** or **By Protocol**.

The list is sorted alphabetically and numerically for any of the choices.

To remove an extraction set from the project

To delete an extraction set from Project Explorer

1 Select an extraction set in Project Explorer.

You can select only one extraction set in Project Explorer.

2 Right-click Project Explorer, and click **Delete**.

The extraction set is immediately deleted.

Or

Click the Delete Extraction Set(s) icon, or click **Edit > Delete Extraction Set(s).**

If an extraction set in Project Explorer is selected, a message appears to ask if you want to delete the single set in Project Explorer. Click **Yes**.

If an extraction set or sets in the Extraction Set Configuration table is selected, a message appears to ask if you want to delete the extraction sets in the table.

To delete an extraction set or sets from the Extraction Set Configuration table

1 Select an extraction set or sets in the Extraction Set Configuration table.

You can select more than one by holding down the **Ctrl** key while you click additional extraction sets.

2 Right-click the selected extraction sets, and click **Delete Extraction set(s)**.

The extraction sets(s) are immediately deleted
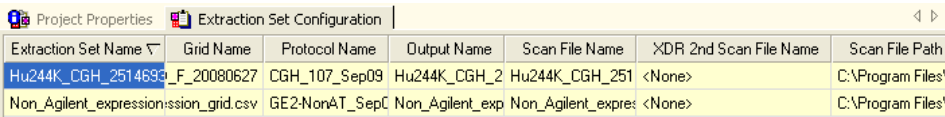
Or

Click the **Delete Extraction Set(s)** icon, or click **Edit > Delete Extraction set(s).**

If an extraction set or sets in the Extraction Set Configuration table are selected, a message appears to ask if you want to delete the extraction sets in the table. Click **Yes**.

If different extraction sets are selected in both Project Explorer and the Extraction Set Configuration table, a message appears to ask if you want to delete the extraction set in Project Explorer. Click **Yes**. To delete the selected sets in the Extraction Set Configuration table, you must repeat step 2.

To display/edit the extraction Set Configuration table

1 Click the Extraction Set Configuration tab.

2 Drag the column marker on the header to expand each cell.

Figure 14   Extraction Set Configuration table

The Extraction Set Configuration tab is a table that lets you review your extraction sets all in one place.

Extraction Set Name   The name of the extraction set. This name is automatically set to be the same as that of the scan file.

Grid Name   The name of the grid template or grid file belonging to the extraction set.

- Click the cell and click the arrow to add or change this template or file from this column.
- Double-click the cell to view the Grid Template Properties dialog box.

Protocol Name   Protocol used with the grid template or project and belonging to the extraction set.

- Click the cell and click the arrow to add or change the protocol from this column.
- Double-click the cell to view the Protocol Editor.

Output Name   The name of the results file. This name is set to the name of the extraction set name with the name of the protocol added.

Scan File Name   The name of the scan file that belongs to the extraction set.

- Double-click this name to see the image in the scan file.

Scan File Path   The directory that contains the scan file.

Working with grid templates/files

A microarray design is the layout of probes on a single glass slide with a particular format. Each array design contains the biological and control probe groups, as well as a required Agilent quality control grid (grid template). When working in Feature Extraction, the Grid Template Browser displays the array designs from which the grid templates are derived. Designs that are displayed in the Grid Template Browser were imported manually from a hard disk location, or downloaded manually or automatically from the Agilent eArray server.

To manage grid templates with Agilent Genomic Workbench

Feature Extraction 10.10 and Agilent Genomic Workbench Standard Edition use the same common database and file storage. Microarray design files (GEML files) created or downloaded by the eArrayXD module of Agilent Genomic Workbench are also used by Feature Extraction as grid templates for extraction.

There are 3 ways for you to set up/obtain a grid template (from an array design) using the Agilent Genomic Workbench shared database:

- Automatically find Agilent catalog array designs or your custom array designs from the eArray Web site under your workgroup account
  - When you set up an extraction, Feature Extraction first checks if the array design file for this AMADID (**A**gilent **M**icro**A**rray **D**esign **ID**entifier) is in the Agilent Genomic Workbench common data storage. If any user in your workgroup previously downloaded or worked with this array design, the design file may already be present.

- If you selected in your eArray settings to "Use eArray server during extraction", Feature Extraction checks on the eArray Web site for any recent array design file update for this AMADID. See "To automatically update required and existing array designs/grid templates" on page 86. If a newer version of the design file exists on the eArray Web site, or the design file is not present in the Agilent Genomic Workbench common data storage, Feature Extraction automatically downloads the design file and uses it for extraction. After the file is downloaded, Feature Extraction automatically sets up a job for Agilent Genomic Workbench to add the corresponding design information to the database, which can later be used to analyze data with one of the Agilent Genomic Workbench analysis modules.

- Use Online Update in the Grid Template Browser to manually search for and import or update array design files from the eArray server.

- Add the array design/grid template file manually

  Array designs that cannot be found on the eArray Web site as a catalog design or under your workgroup account (for example, designs you have through private collaborations, or early-access Agilent catalog designs) must be imported manually. See "To add or remove a grid template" on page 84.

  After a grid template/array design is added manually, the grid template is displayed in the Grid Template Browser. It is also displayed in Agilent Genomic Workbench, in the Design Data pane of the Navigator, in the Custom Designs folder. Routine annotation update checks are not performed on these designs because they are not present on the eArray Web site.

How dye normalization lists are associated with array designs/grid templates

Array designs created on eArray.com have optional dye normalization list files associated with them. When Feature Extraction downloads or updates an array design (grid template), if an associated dye normalization list exists for that design, it is automatically imported and saved with the design (grid template) in the database. Once an array design is created in eArray, the dye norm list is fixed. Annotation updates can happen, yet the dye norm list of probes will never change.

If you add an array design/grid template file manually, Feature Extraction does not automatically associate a dye normalization gene list file with the design. You must associate the dye normalization gene list with the array design/grid template using the Grid Template Properties. See "To change the dye normalization gene list in the grid template" on page 92.

The following table shows how rules are applied when Feature Extraction updates array designs (grid templates) that have dye normalization gene lists. This table assumes that **Use eArray server during extraction** and **Check for updates of grid templates** options are selected in Tools>eArray Settings.

Table 2   Dye Normalization List Rules

Grid template/design has dye normalization list association?	Protocol use dye norm list selection	Action	Result
Grid template not in database-No	Automatically Determine	Run a feature extraction	Grid template/design is automatically downloaded from eArray and used for extraction. Dye normalization probe selection method set in the Correct Dye Biases step of the protocol is used.
Grid template not in database - Yes	Automatically Determine	Run a feature extraction	Grid template/design is automatically downloaded from eArray, with associated dye norrmalization list, and used for extraction.
Old version - Yes New version - No	Automatically Determine	Run a feature extraction	The dye normalization gene list from the older version is automatically associated with the new grid template version, which is then used for extraction.
Old version - No New version - Yes	Automatically Determine	Run a feature extraction	The dye normalization list and the new grid template version are used.
Old version - No New version - No	Automatically Determine	Run a feature extraction	Feature Extraction uses the dye normalization probe selection method set in the Correct Dye Biases step of the protocol.

Table 2   Dye Normalization List Rules

Grid template/design has dye normalization list association?	Protocol use dye norm list selection	Action	Result
New version - Yes	Automatically Determine	Create a new dye normalization list and associate it in the grid template properties Run a feature extraction	The new dye normalization list is used for extraction, however, the original dye normalization list is still available.
New version - Yes	True	Run a feature extraction	The associated dye normalization list associated with the design is used.
Old Version - No New Version - No	True	Run a feature extraction	The extraction fails because no dye normalization list is found
New Version - Yes	False	Run a feature extraction	Feature Extraction uses the dye normalization probe selection method set in the Correct Dye Biases step of the protocol.

Setting up and Changing Grid Templates and Files

To show or hide the Grid Template Browser

When you first open the Feature Extraction software after installation, the Grid Template Browser appears on the right-hand side of the main window.

If the Browser is closed, you can re-open it:

- Click **View > Grid Template Browser**.

To add or change a grid template to an extraction set

If you add or change an array design/grid template that does not match the design file for an Agilent image, the software highlights the template in orange in the Extraction Set Configuration tab to warn you. Extraction can still be run on this set, if you did not select for Feature Extraction to automatically assign a grid template, but gridding or annotation may be incorrect.

If you want to add an array design/grid template or use a different grid template with the image file in the extraction set, follow these instructions:

1 Select a grid template from the list of Grid Templates.

2 Drag and drop the grid template onto the extraction set.

The new grid template appears in the extraction set.

To add or change a grid file to an extraction set

Although you can add a grid file for Agilent microarrays, you must add or create a grid file for non-Agilent microarrays.

1 In the Project Explorer, right-click the second line of the extraction set, and click **Select grid file** from the menu.

2 Select the .csv file that you want to use with the image file, and click **Open**.

To add or remove a grid template

To add an array design/grid template to the Grid Template Browser from a file:

1 Click **Tools > Grid Template > Add.**

Or

Right-click the Grid Template Browser, and click **Add.**

2 In the Add Grid Template dialog box, locate and select an array design file or grid file, and click **Open**.

Grid templates are created from either array design files or grid files. They do not exist as objects in the file system.

NOTE When you add a grid template (array design) in Feature Extraction, the design is available in Agilent Genomic Workbench as well.

To remove a grid template/array design from the Grid Template Browser:

1 Click **Tools > Grid Template > Remove**.

Or

Right-click the Grid Template Browser, and click **Remove**.

2 Click **Yes** when asked if you're sure you want to remove the grid template from the browser.

> **NOTE**   When you remove a grid template (array design) from the Grid Template
> Browser, it is not removed from the Agilent Genomic Workbench
> database.

To open grid mode from an extraction set

If automatic grid placement fails during extraction, you may
want to create and use a grid file in place of the grid
template, or edit the grid file, depending on which is
associated with the extraction set. Or, you may just want to
"fine tune" a grid placement. You can more easily access grid
mode from Project Explorer in configuration mode than from
the image display.

For non-Agilent images, you can
also access grid mode from the
image display. See "Step 1. Start
grid mode from the non-Agilent
image file" on page 131.

1 In Project Explorer, right-click the grid template or grid
file in the extraction set.

2 Click **Open Current Extraction in Grid Mode**.

The image file for the extraction set appears in grid mode,
ready for gridding.

See Chapter 3, "Creating Grid Files" in this guide to learn
how to set up or edit a grid file.

To update array designs/grid templates online (via Agilent eArray)

As new genomic information becomes available, Agilent
updates its array design files (grid templates). These updates
are available for downloading through the Agilent eArray
Web site.

There are two different ways to load new array designs/grid
templates that contain the latest annotation available on
eArray into the Feature Extraction database.

• Automatic updates of required and existing array
designs/grid templates.

You can set up Feature Extraction to automatically update array designs/grid templates that are required by the extraction sets, but are not available in the database. During the extraction run, the software will log into the eArray server using your eArray user name and password and download, install, and use the array designs/grid templates to process the extraction sets. See "Setting up eArray login for automatic updates."

• Manual updates of new and existing array designs/grid templates.

You can manually update existing array designs/grid templates with the latest annotation or add new array designs/grid templates that are not in the database. You must provide the user name and password of your eArray account.

To automatically update required and existing array designs/grid templates

1 Select **Tools > eArray Settings**.

The dialog box shown in Figure 15 appears.
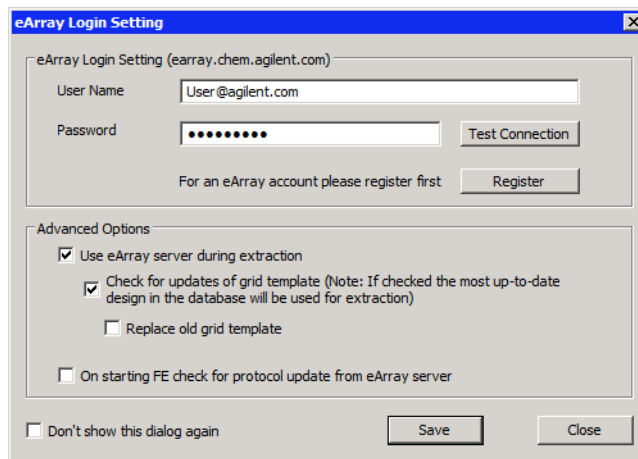
Figure 15   eArray Login Setting window

2 Type your **User Name** and **Password** in the eArray Login Setting window, if you already have an existing eArray account.

Hint: The user name is the e-mail address you provided when you created an eArray account.

If you do not have an eArray account, you must first register for one. Click **Register** to start the registration process. This will take you to the Agilent eArray registration Web site where you can request a free eArray account. Follow the instructions provided on the eArray Web site to complete the registration.

3 Click **Test Connection**. The message "Login Successful!!!" pops up if the connection to the eArray server is successful. If you get the message "Login denied", you should check that the user name and password you typed are correct.

4 In the Advanced Options, select the appropriate boxes:

• **Use eArray server during extraction**

You must select this box to let Feature Extraction log into eArray server during the extraction run to download, install, and use required grid templates (array designs) that are missing in the Feature Extraction database.

• **Check for updates of grid template**

Select this box if you want Feature Extraction to download updates to existing array designs/grid templates already in the database. Existing designs/grid templates in the database are not replaced, unless you select **Replace old grid template**.

• **Replace old grid template**

Select this box to automatically replace old array designs/grid templates in the Grid Template Browser with the latest ones from eArray server.

NOTE	The existing array design files will not be overwritten in the Agilent Genomic Workbench; the new version of that design is added to the database without deleting the previous version. However, Feature Extraction uses only the latest version of that design/grid template, unless you designate the older design/grid template version in the extraction set and clear the Use eArray server during extraction check box.

5 To save the eArray login settings, click **Save**.

You will get a message "Setting updated successfully". Click **OK** to close the message.

6 Click **Close** to exit the eArray Login Setting window.

To manually update new and existing array designs/grid templates

1 Right-click anywhere in the Grid Template Browser, and click **Online Update**.

Figure 16 appears with the design IDs and version dates of the array designs/grid templates that currently exist in the Feature Extraction database.

More than one version of the array design/grid template can reside in the database. The update function lets you choose to add the latest version from eArray to already existing versions or to overwrite the most recent version already in the database.
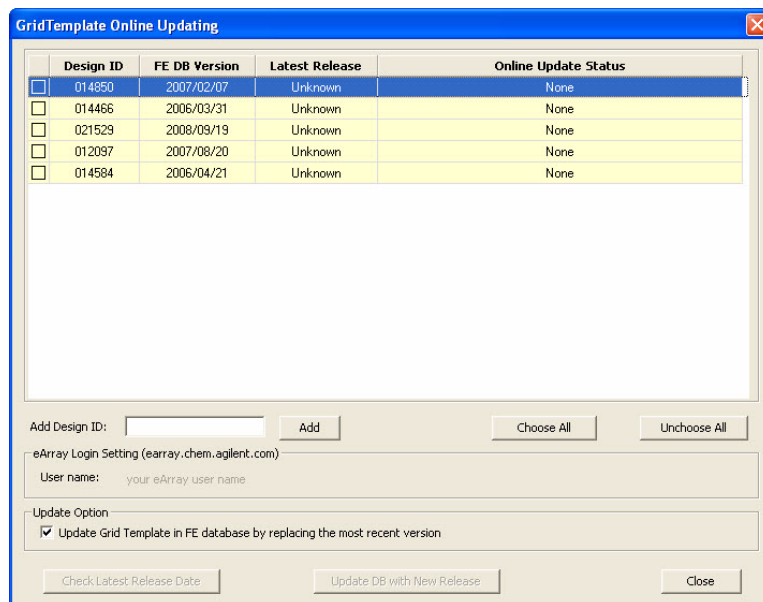
Figure 16   Grid Template Online Updating window

2 Click **Choose All** or select only the Design IDs that you want to check for updates, and the bottom buttons are activated.

3 If you want to add a Design ID not in the list, type the ID in the Add Design ID field, and click **Add**.

If you don't see your user name in the User name field, you must set up your eArray login first. See "To automatically update required and existing array designs/grid templates" on page 86 for details on how to set up your eArray login.

4 To check the release dates, click **Check Latest Release Date**, Or

5 To add the most recent array design/grid template to the database, click **Update DB with New Release**.

If you want to add the latest release(s) to the Feature Extraction database without overwriting the most recent version, *clear* the check box for **Update Grid Template in FE database by replacing the most recent version**.

NOTE	The existing design files are not overwritten in the Agilent Genomic Workbench; the new version of that design is added to the database without deleting the previous version. However, Feature Extraction uses only the latest version of that design/grid template, unless you clear the Use eArray server during extraction check box in the eArray settings. See "Setting up eArray login for automatic updates" on page 36.

To display or change grid template properties

You can only change grid template properties such as default protocols and associated dye normalization lists. Array design properties (such as geometry) cannot be changed.

1 Double-click the grid template/array design whose properties you want to display or change

2 Display the grid template information.

You can display all of the grid information. You can also change the default protocol (Agilent or non-Agilent 2-color, CGH, ChIP, or miRNA), the default 1-color protocol, and the dye normalization gene list.

Figure 17   Grid Template Properties window

To add or change the default protocol in the grid template

1 Double-click the grid template whose protocol you want to add or change.

2 Click the cell to the right of Default protocol, and select the protocol from the list.

If you don't see the protocol you want from the list, you must import the protocol into the database first. See "To import or export a protocol to or from the database" on page 102.

To add or change the default one-color protocol in the grid template

Agilent design files and grid templates are the same for 2-color and 1-color or 2-color experiments. To make sure that the software can use a 1-color protocol with 1-color

data, both 2-color and 1-color default protocols are specified. The software uses the 1-color protocol when the 1-color scan file is loaded.

1 Double-click the grid template whose protocol you want to add or change.

2 Click the cell to the right of Default OneColor protocol, and select a 1-color protocol from the list.

If you don't see the protocol you want from the list, you must import the protocol into the database first. See "To import or export a protocol to or from the database" on page 102.

To change the dye normalization gene list in the grid template

If the protocol specifies use of a dye normalization gene list and the extraction set includes a grid template, you create or select the gene list in the Grid Template Properties window.

1 Double-click the grid template whose dye normalization gene list you want to add or change.

2 Click the arrow to the right of the cell next to Dye Normalization Gene List.
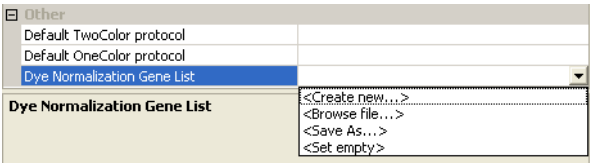
Figure 18 Dye Normalization Gene List options

3 Select one of the following options:

Dye Normalization Gene List   This is blank if no DyeNorm Gene Lists are available or if you select Set Empty.

If you previously created an internal dye normalization gene list, or selected an external gene list, select a name from this list. Once selected, you can double-click the gene list name to open the DyeNormList Editor to display or edit the list.

Create New   Select this option if you want to create a new internal list from the existing probes. See "To create a new internal dye normalization gene list for a grid template" on page 93.

Browse file   Select this option to browse and select an external gene list for the default list. External dye normalization lists are not imported into the database.

Save As   Saves the selected dye normalization gene list as an external list. When saving, you can change the file name of the external list.

Set Empty   Select this option if you do not want Feature Extraction to use a dye normalization gene list with this grid template.

To create a new internal dye normalization gene list for a grid template

1 Double-click the grid template for which you want to create a dye normalization gene list.

2 Click the cell to the right of Dye Normalization Gene List.

3 Click the arrow and click **Create New**.

NOTE   You can create a dye normalization gene list using the Gene/Probe View, or using the Chromosome View, but not both.
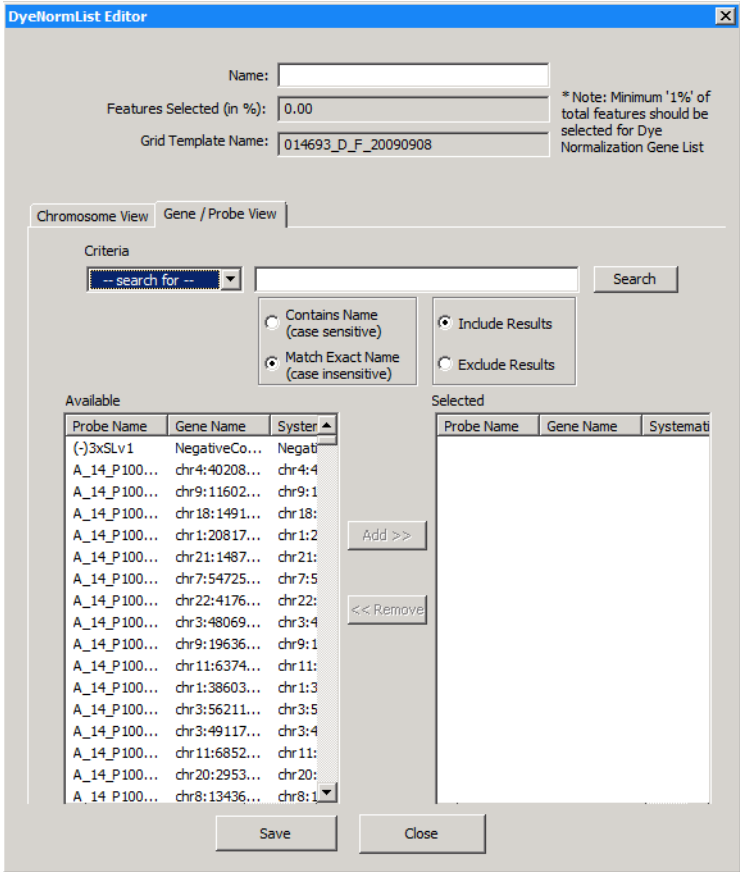
Figure 19   DyeNorm List Editor—Gene/Probe View

Add genes to list by search criteria

To streamline the process of selecting genes for the list, you can search for genes according to Probe Name, Gene Name or Systematic Name. SNP probes are not included.

1 If necessary, click the **Gene/Probe View** tab.

2 Click the box next to **Name** and type a name for the dye normalization list.

3 Under Criteria, click **Search for**, and then select **Probe Name**, **Gene Name** or **Systematic Name** as the type of name for the search.

4 Select how you want to use the name in the search. You can search according to the exact name, part of the name, or using a regular expression.

5 Type the name or part of the name for the search.

6 Select to include or exclude results.

7 Click **Search**.

Name   Type the name for the dye normalization list. The list you create will be saved as an internal dye normalization list with this name.

Features Selected (in %)   The dye normalization list must contain at least 1% of the total features in the array. This indicates the % of the features currently selected for the list.

Grid Template Name   This shows the grid template with which you are associating this dye normalization list.

Contains Name (case sensitive)   If selected, you can search for part of the name, or you can use a regular expression. The case must match exactly. Regular expressions give you advanced search capabilities. For example,

   • If you want to search all probes you can type the regular expression as "."(dot) and it will search all the probes.

NOTE	For information on using regular expressions for search, see the following article: http://msdn.microsoft.com/en-us/library/k3zs4axe%28VS.80%29.aspx.

Match Exact Name (case insensitive)   If selected, you must type the exact probe name, gene name or systematic name, (depending on the type of name you selected in step 1.) For this search, the case does not need to match.

Include Results   If selected, all the results found are transferred from the Available Genes on Array list to the Selected Genes from Array list.

Exclude Results   If selected, all the results *not* found are transferred from the Available Genes on Array list to the Selected Genes from Array list.

Add chromosomes to list

1 Click the **Chromosome View** tab.
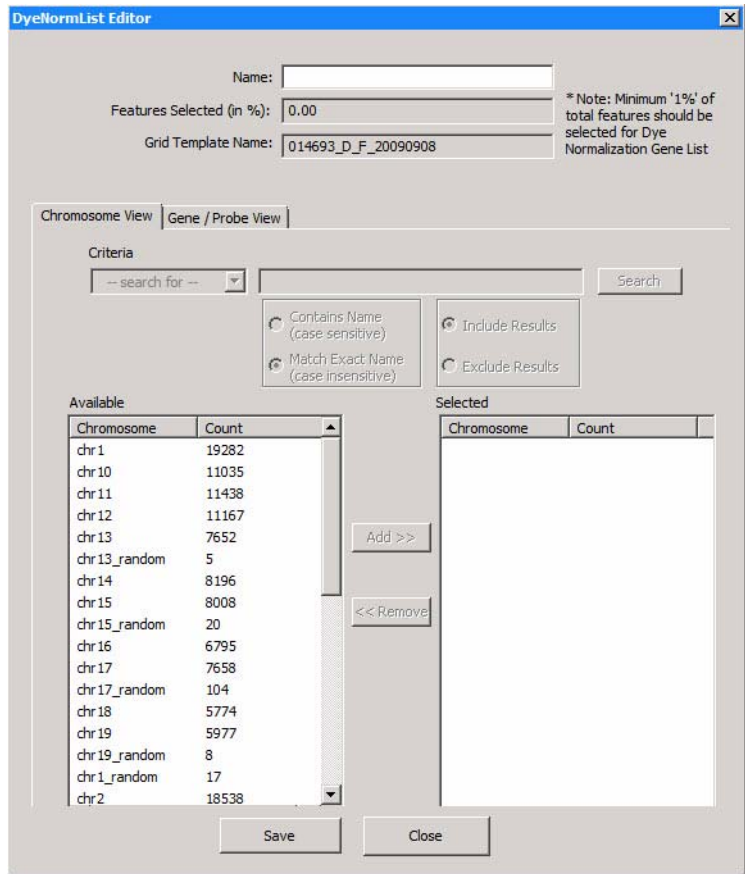
Figure 20   DyeNorm List Editor—Chromosome View

2 Select the chromosome(s) from the Available list, and click **Add>>**. See "Add/remove genes or chromosomes to list interactively" on page 97.

Add/remove genes or chromosomes to list interactively

1 Select the gene(s) or chromosome(s) of interest from the Available list.

For multiple selection, hold down the **Shift** key and click the first name and then the last name or hold the **Ctrl** key and click for non-contiguous names.

2 Click **Add >>**, or

Click **<<Remove** to transfer genes from the Selected list to the Available list.

If you attempt to transfer a large number of genes or chromosomes from list to list, the process can take time.

Save dye normalization gene list to internal file

You may have to refresh the screen in order to see the name of the new list in the Grid Template Properties window.

If you want to save the internal dye normalization list as an external file, see "To export an internal dye normalization gene list" on page 98.

1 Type a name for the dye normalization list in the Name field.

2 Click **Save**.

When you click **Save**, a list is created using the Name specified, and is placed in the internal list available to the grid template.

To edit an internal dye normalization list

1 Double-click the grid template whose dye normalization gene list you want to change.

2 Click the cell to the right of Dye Normalization Gene List.

3 Select an existing gene list.

4 Double-click the list to bring up the **DyeNormList Editor**.

5 Add and remove any genes or chromosomes that you want.

Once an internal dye normalization gene list is created, you cannot change it. You must save changes using a different name.

6 Click **Save As**.

To import external dye normalization list to create a new internal list

1 Double-click the grid template whose dye normalization gene list you want to add or change.

2 If you want to create an internal list from an external .txt file, click the cell next to **Dye Normalization Gene List,** and click the down arrow.

3 Click **Browse file**, select the external .txt file and click **OK**.

This file must be in a special format: 3 columns and a carriage return with Probe Name, Gene Name and Systematic Name listed in that order. The file must not have a row of column headers.

4 Click the down arrow again**,** and select imported gene list as the default list.

If you need to make changes to the gene list, see "To edit an internal dye normalization list" on page 97.

To export an internal dye normalization gene list

After you create an internal dye normalization gene list file, you can export it as a .txt file to any folder you choose.

1 Double-click the grid template whose dye normalization gene list you want to export.

2 Click the down arrow to the right of the cell next to Dye Normalization Gene List, and select the internal gene list.

3 Click the down arrow again, and click **Save As**.

4 Select a folder you want to save the file to, rename the file, if necessary, and click **Save**.

The file is saved as an external .txt file.

Working with protocols

To move custom protocols from version 10.7 to 10.10

In order to use custom protocols you created with Feature Extraction 10.7 in Feature Extraction 10.10, you must first export those protocols using Feature Extraction 10.7. Exported protocols are saved as .xml files. You can then import the saved Feature Extraction 10.7 protocol files to Feature Extraction 10.10. See "To import or export a protocol to or from the database" on page 102.

To show or hide the FE Protocol Browser

When you first open the Feature Extraction software after installation, the FE Protocol Browser appears on the right-hand side of the main window.

If the Browser has been closed, you can re-open it:

- Click **View > FE Protocol Browser**.

To add a protocol to an extraction set

By default, the program will automatically determine the protocol to use for the extraction. If you want to manually add a protocol to an extraction set, follow these instructions:

1 Click to select a protocol in the FE Protocol Browser.

2 Hold down the mouse button and drag and drop the protocol onto the third line in the extraction set.

The new protocol appears in the extraction set.

To change the protocol used in an extraction set

If you want to use a different protocol than that associated with the grid template or project, follow these instructions:

1 Select a protocol from the FE Protocol Browser.

2 Hold down the mouse button and drag and drop the protocol onto the extraction set.

The new protocol appears in the extraction set, and replaces the previous protocol.

To display or change protocol properties

1 Double-click a protocol in the FE Protocol Browser to open the Protocol Editor.
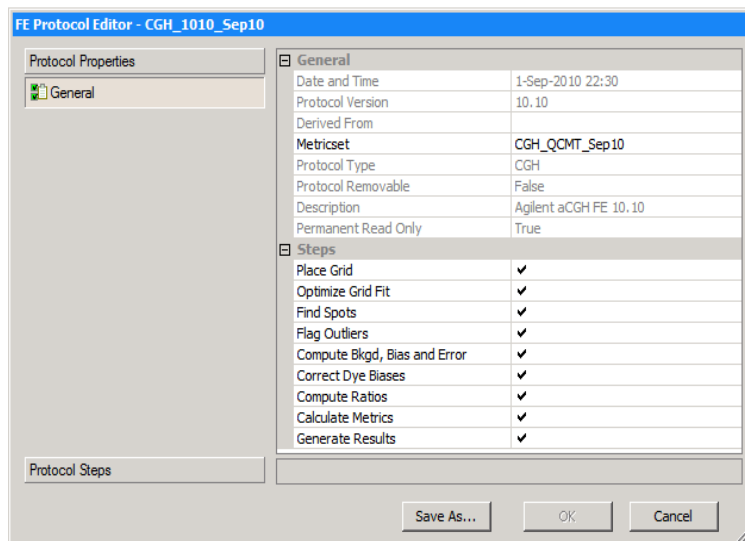
Figure 21   FE Protocol Editor—Protocol Properties

2 Click the **Protocol Properties** button which shows the General tab.

Protocols and QC metric sets are linked. Each protocol has a metric set assigned to it. When Feature Extraction updates protocols from the eArray server, it also updates the corresponding metric sets.

You can view information about the protocol. Under the General section, you can change the QC metric set that is associated with the protocol. Under the Steps section, you can remove protocol steps from the extraction run.

3 To change the QC metric set assigned to the protocol, click the cell to the right of Metricset, and select a QC Metric Set or Set empty.

4 To remove a protocol step from the run, clear the check box next to the step.

5 Click **Save As** to save the changes with a new protocol name.

See Chapter 4, "Changing Protocol Settings" for more details.

To import or export a protocol to or from the database

To import a protocol to the database:

1 Click **Tools > FE Protocol > Import**.

Or

Right-click the FE Protocol Browser, and click **Import**.

2 Select a protocol from the list, and click **Open**.

To export a protocol from the database:

1 Click **Tools > FE Protocol > Export**.

OR

Right-click the FE Protocol Browser, and click **Export**.

2 Select a protocol from the list, and click **Save**.

A copy of the protocol is exported, but the protocol remains in the database.

To remove a protocol from the database

The **Derived from** message can help you keep track of user-created protocols. If the protocol is Agilent-created, the message indicates this. If the protocol is user-created, the message lists the original Agilent protocol from which the new protocol was derived.

1 Click **Tools > FE Protocol > Remove**.

Or

Right-click the protocol in the FE Protocol Browser, and click **Remove**.

2 Click **Yes** when asked to make sure that you want to remove this protocol.

Agilent-created protocols cannot be removed from the database.

Working with QC metric sets

By default, the program calculates a set of default statistics that are displayed in the QC report. QC metric sets give you additional statistics for evaluating the Feature Extraction process on your microarrays. These statistics also appear in the QC report and in the Stats table of the text results file.

QC metric sets are linked with protocols. Each protocol has a metric set assigned to it. When the protocol is used by the software, the QC metric set is also used to generate metrics about the extraction set. During updates of protocols from the eArray server to the Feature Extraction database, the corresponding QC metric sets are also updated.

Agilent-created QC metric sets are installed with the Feature Extraction software and can be updated from the eArray Web site. You can also create your own QC metrics sets using the Quality tools in Agilent Genomic Workbench. When you create a QC metric set in the Quality tab of Agilent Genomic Workbench, it will automatically be available in the QC Metric Set Browser of Feature Extraction. See "To import or export a QC Metric Set to or from the database" on page 105.

If you wish to use your custom QC metric set with a protocol, you must associate it to the protocol. See "To associate a QC metric set with a protocol" on page 105.

To move custom QC metric sets from version 10.7 to 10.10

If you created custom metric sets in Feature Extraction 10.7, and you want to use them in Feature Extraction 10.10, you must first export them from Feature Extraction 10.7. This saves each QC metric set in an .xml file. You must then import the QC metric sets in Feature Extraction 10.10. See "To import or export a QC Metric Set to or from the database" on page 105. If you used the QC Chart Tools program, provided with Feature Extraction 10.7, to create custom QC metric sets and queries, see the Agilent Genomic

Workbench *Quality Tools User Guide* for information on how to move custom metric sets and queries from QC Chart Tools 10.7 to Agilent Genomic Workbench Standard Edition.

To show or hide the QC Metric Set Browser

This Browser contains the most recent Agilent QC metric sets after the software is installed. In addition, it shows custom QC metric sets that were created in the Quality tab of Agilent Genomic Workbench. To show or hide the QC Metric Set Browser:

• Click **View > QC Metric Set Browser**.

To associate a QC metric set with a protocol

You associate a QC metric set with a protocol in the FE Protocol Editor.

1 Double-click on the protocol to associate a QC metric set with. This opens the FE Protocol Editor.

2 Click the cell next to Metricset, and click the down arrow.

3 Select a QC metric set from the list.

To import or export a QC Metric Set to or from the database

To import a QC Metric Set to the database:

1 Click **Tools > QC Metric Set > Import**.

Or

Right-click the QC Metric Set Browser, and click **Import**.

2 Select a QC Metric Set from the list, and click **Open**.

To export a QC Metric Set from the database:

1 Click **Tools > QC Metric Set > Export**.

Or

Right-click on a QC metric set in the QC Metric Set Browser, and click **Export**.

2 Select a QC metric set from the list, and click **Save**.

A copy of the QC metric set is exported, but the metric set remains in the database.

To remove a QC metric set from the database

Default Metric Sets and Protocols that are loaded to the database when you install the software are not removable.

1 Click **Tools > QC Metric Set > Remove**.

Or

Right-click the QC Metric Set Browser, and click **Remove**.

2 Click **Yes** when asked to make sure that you want to remove this metric set.

To view metric set properties

1 In the QC Metric Set Browser, double-click a metric set you wish to view.

For details on the logic used for metric set evaluation, see the Feature Extraction 10.10 Reference Guide.

The QC Metric Set Properties for the selected metric set is displayed. Names of all the metrics included with this metric set are displayed, along with threshold values, if provided. For details on default metric set properties, see the *Feature Extraction 10.10 Reference Guide.*
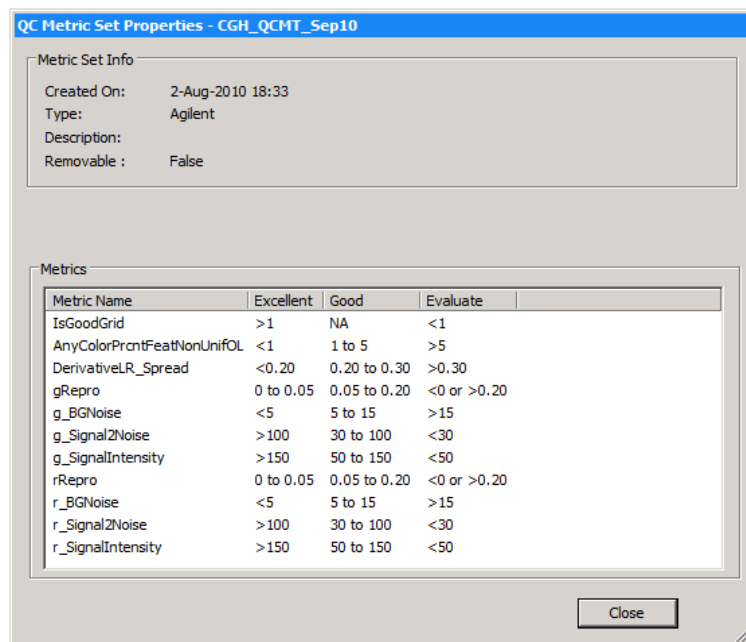
Figure 22   Metric set properties

2 Click **Close** to close the dialog box.

Running Feature Extraction

The following criteria must be met before you can run Feature Extraction on a project.

- If there are extraction sets in the project that contain a red X or (if the eArray connection is good) a lightning bolt, you must set up eArray login to let Feature Extraction automatically the download, install, and use grid templates, protocols, and QC metric sets that are missing from the database.

- Internet connection is required during extraction, if Feature Extraction is expected to log into eArray server to get the missing grid templates, protocols, and QC metric sets.

To start Feature Extraction

1 Click the **Project Run mode On/Off** icon in the toolbar, or click **Project > Start Extracting**.

If you did not save the project, a message appears that asks if you want to save the project. You must save the project in order to run Feature Extraction.

2 Click **Yes**.

3 Click **Save** if you want to save the project to the default FE Project N.fep name, or type a new name, and click **Save**.

During extraction, information on what steps are performed is displayed in the Running Monitor at the bottom of the window. A Project Run Summary report is generated in the Summary Report tab when the extraction is complete.

To stop Feature Extraction

- Click the **Stop Extracting** icon, or click **Project > Stop**.

To create a new project with remaining extraction sets if run fails

If a run fails due to a gridding problem, a message appears and provides an easy way to create a new extraction set containing the failed extraction sets. See "Step 1. Create a new project for failed extraction sets" on page 127 for details.

If a run fails for whatever reason, you can create a project containing the failed or not yet processed extraction sets left over from the previous project run. The following requirements must be met:

• Project must be a Standard FE project.

• Project must still be in the Run mode.

• Project has just stopped extracting and contains failed extraction set(s) or ones not yet run.

1 Click **Project > Create Project for Leftover Set(s)**.

A message appears asking if you want to switch back to Config mode and continue.

2 Click **Yes**.

Feature Extraction creates a new project with all unfinished extraction sets from the current project. Feature Extraction also closes the project and starts the new project in its Config mode.

The name for the newly created project is "Parent project name_2".

You must save the new project before you can extract or exit the project.

Displaying results

An Agilent multiplex array is one that has multiple Hyb/Wash chambers for separate samples on a single slide. These were formerly referred to as "multipack" arrays.

A Feature Extraction run on any image produces the following files:

- A Project Run Summary report .rtf file
- Text result .txt files (generated per microarray)
- For Agilent arrays, a .pdf QC report (generated per microarray) or .html QC report (if you are running with a temporary license.
- A .shp visual result file

NOTE	For Agilent multiplex arrays, text & QC reports are generated for each subarray, but just one .shp and Project Run Summary is produced per extraction of the image.

This section describes how to view these results.

To display the text result file in Microsoft Excel

The result files are named with the following format:

Single pack: extraction set name_protocol name.

Multiplex (formerly known as Multipack): extraction set name_protocol name_pack number, indicated by row number.

1 Go to the folder specified for containing the results.

2 Right-click the text results file (protocol name on end), and click **Open with**.

3 Click **Microsoft Excel**.

The text results will open in the spreadsheet. The following figure shows all three tables (FEPARAMS, STATS, and FEATURE) in the file. You can also choose to create a different text file for each table. See "To select to generate a single file for the text output" on page 255 for more information.

TYPE	text	text	text	integer	text	float	float	text	integer	text	text	integer	integer	integer
FEPARAM	Protocol_I	Protocol_I	Scan_Sca	Scan_Nur	Scan_Dat	Scan_Mici	Scan_Mici	Scan_Orig	Scan_Nur	Grid_Nam	Grid_Date	Grid_Num	Grid_Num	Grid_Num
DATA	CGH_105	######	Agilent Te	2	######	3	3	c121ba31	1	021529_D	######	1	1	1068
*														
TYPE	float	float	float	integer	integer	float	float	float	integer	integer	float	float	integer	float
STATS	gDarkOffs	gDarkOffs	gDarkOffs	gDarkOffs	gSaturatio	rDarkOffs	rDarkOffs	rDarkOffs	rDarkOffs	rSaturation	gAvgSig2E	rAvgSig2B	gNumSatf	gLocalBG
DATA	24.3684	24.3684	1.99337	1048576	65510	24.5267	24.5267	2.32225	1048576	65510	1.75806	1.28801	8	11.5053
*														
TYPE	integer	integer	integer	integer	integer	text	text	float	float	float	float	float	float	float
FEATURE	FeatureNu	Row	Col	SubTypeM	ControlTyr	ProbeNan	Systemati	PositionX	PositionY	LogRatio	LogRatioE	PValueLoi	gProcessi	rProcesse
DATA	1	1	1	0	1	HsCGHBr	HsCGHBr	606.969	388.687	5.45E-02	6.17E-02	3.77E-01	5.56E+03	6.31E+03
DATA	2	1	2	66	1	DarkCorn	DarkCorn	628.333	388.609	0.00E+00	6.20E-01	1.00E+00	3.25E+01	3.35E+01
DATA	3	1	3	66	1	DarkCorn	DarkCorn	649.5	388.676	0.00E+00	6.20E-01	1.00E+00	3.25E+01	3.35E+01
DATA	4	1	4	0	0	A_16_P01	chr5:0750	670.509	388.632	-1.85E-02	6.70E-02	7.82E-01	7.81E+02	7.48E+02
DATA	5	1	5	0	0	A_16_P01	chr4:1145	691.426	388.926	4.03E-02	7.10E-02	5.71E-01	5.49E+02	6.02E+02
DATA	6	1	6	0	0	A_16_P16	chr4:0413	712.577	388.923	-3.54E-02	6.62E-02	5.93E-01	8.69E+02	8.01E+02
DATA	7	1	7	0	0	A_16_P0C	chr2:2077	733.673	388.909	-2.02E-02	6.23E-02	7.46E-01	1.99E+03	1.90E+03
DATA	8	1	8	0	0	A_16_P36	chr7:0777	755.158	388.684	-3.58E-02	6.69E-02	5.92E-01	8.04E+02	7.41E+02
DATA	9	1	9	0	0	A_16_P0C	chr1:1500	776.038	388.865	5.78E-02	6.35E-02	3.63E-01	1.22E+03	1.40E+03
DATA	10	1	10	0	0	A_16_P20	chr17:071	797.5	388.722	7.14E-02	6.41E-02	2.65E-01	1.08E+03	1.28E+03
DATA	11	1	11	0	0	A_14_P13	chr5:0986	818.75	388.607	1.77E-02	6.38E-02	7.82E-01	1.14E+03	1.19E+03
DATA	12	1	12	0	0	A_16_P02	chr10:024	839.825	388.614	6.04E-02	7.01E-02	3.89E-01	5.64E+02	6.48E+02
DATA	13	1	13	0	0	A_16_P36	chr9:1294	860.903	388.403	2.80E-02	7.35E-02	7.03E-01	4.83E+02	5.15E+02
DATA	14	1	14	0	0	A_16_P0C	chr1:1490	882.317	388.733	4.42E-02	6.25E-02	4.79E-01	1.80E+03	2.00E+03
DATA	15	1	15	0	0	A_16_P36	chr2:1976	903.154	388.846	1.83E-03	6.27E-02	9.77E-01	1.56E+03	1.57E+03
DATA	16	1	16	0	0	A_16_P17	chr6:0804	924.333	388.741	-1.08E-01	6.48E-02	9.54E-02	1.24E+03	9.66E+02
DATA	17	1	17	0	0	A_16_P18	chr7:0829	945.407	389.237	-5.00E-02	6.25E-02	4.23E-01	2.01E+03	1.79E+03
DATA	18	1	18	0	0	A_18_P15	chr5:0368	966.793	388.948	-3.44E-02	6.24E-02	5.82E-01	1.92E+03	1.78E+03
DATA	19	1	19	0	0	A_16_P39	chr11:006	988.075	388.679	7.13E-02	6.50E-02	2.73E-01	9.03E+02	1.06E+03
DATA	20	1	20	0	0	A_18_P23	chr3:1710	1009.34	389.16	7.80E-02	7.30E-02	2.86E-01	4.67E+02	5.59E+02
DATA	21	1	21	0	0	A_16_P17	chr5:1399	1030.32	388.877	6.44E-02	6.22E-02	3.00E-01	2.30E+03	2.67E+03
DATA	22	1	22	0	0	A_16_P16	chr4:0266	1051.31	388.741	8.57E-03	6.25E-02	8.91E-01	1.74E+03	1.77E+03

Figure 23   Portion of text results file in Microsoft Excel

For some microarrays, the results will not all fit in an Excel spreadsheet (only the first 65536 rows in Excel 2003 and earlier). Results can be viewed in GeneSpring or DNA Analytics.

To display the Project Run Summary Report

When you start the extraction run, the Summary Report tab appears showing the project start time. Once extraction is completed, you can see information about the extraction set in run summary. This summary is automatically saved as a Project Run Summary report that you can access later.

1 Go to the folder containing the project whose report you want to display.

The default directory for Feature Extraction projects is C:\Program Files\Agilent\Agilent Genomic Workbench Standard Edition <version>\FeatureExtraction\ FEProjects.

2 Double-click the file named **YourComputerName_ LastBatchReport.rtf** to display the last saved report for the project.

Or, double-click the file named **FE Project N_timestamp.rtf** to display a summary report for a specific run of the project.

Figure 24   Project Run Summary

You can open the QC Report by double-clicking the blue QC Report link in the Project Run Summary.

Evaluation of grid placement failures

If you see one of these warnings in the Project Summary Report, check the QC report and the .shp file to confirm the success or failure of the grid placement.

• Grid failure error. There are many checks that are protocol dependent. These include number of features not found, number non-uniform local background regions, negative control standard deviation, and %CV of replicated features. Depending upon the thresholds set for these metrics in the protocol, the array may or may not show a grid failure error.

• The average difference between the nominal grid location and the spot centroid is greater than 10 microns.

If the grid location does not fit within the image, the software will stop the extraction and produce an error in the Project Run Summary.

If automated extraction fails for an Agilent image

Several options are available to troubleshoot the failure. See "If automatic gridding for an Agilent image fails" on page 127 for instructions for a successful grid placement and extraction.

If there is a warning that there is no barcode in the MAGE-ML output

Absence of a barcode may be due to several reasons:

• Barcode reader failure

• Agilent scanner cannot read the Agilent microarray barcode for some reason.

• Scan region set up to scan a non-Agilent microarray does not contain the barcode.

For Feature Extraction to load MAGE-ML results files into Rosetta Resolver, they must have a barcode or microarray identifier associated with them. You can add this identifier in the Scan Image Properties dialog box. See "To add or change the New Array Identifier" on page 263.

To display the QC Report

Six types of QC reports can be generated:

- 2-color GE QC report with or without spike-ins
- 1-color GE QC report with or without spike-ins
- CGH_ChIP QC report
- Streamlined CGH QC report
- MicroRNA (miRNA) report
- Chip test report (used by Agilent service personnel for scanner performance verification)

The report generated depends on the protocol used, and is specified in the Generate Results settings for the protocol. See "Changing Protocol Step10: Generate Results" on page 254.

1 Go to the folder specified for containing the results.

2 Double-click the QC Report .pdf or .html file.

The QC Report can be generated in .pdf or .html format. If the Feature Extraction license is a demo license, only an .html file is generated.

You can also access the QC Report from the Project Run Summary. Double-click the blue link next to QC Report. See Figure 24 on page 112.

Figure 25   Streamlined CGH QC Report—Page 1
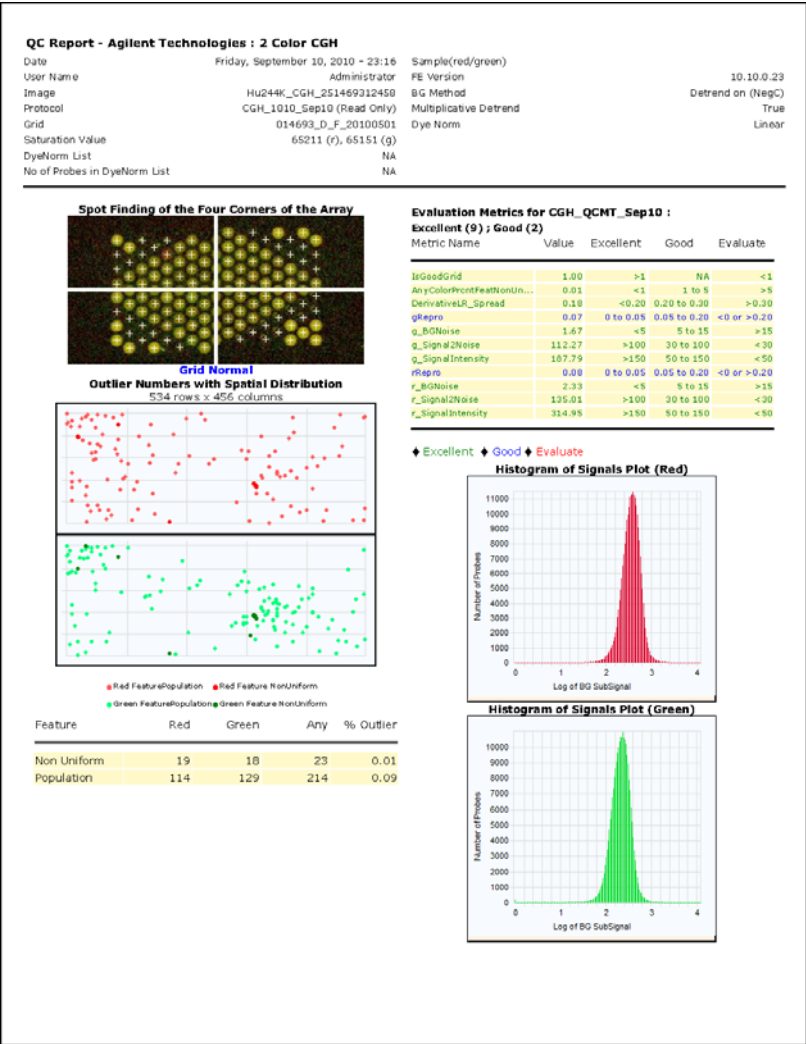
To print the QC Report

If the QC report is open in Internet Explorer, follow these instructions:

1 Click **File > Page Setup**.

2 Make sure that it prints in Portrait mode.

3 Select **A4** size paper.

4 Click **Printer > Properties**, and select for color printing.

5 Exit until you see Page Setup, then close Page Setup.

6 Click **File > Print Preview**.

7 Adjust the margins to display all the images.

8 Click **Print**.

You can alternatively use another Internet browser that has a Shrink to Fit option for printing, such as Mozilla Firefox.

To display visual results

After extraction is completed, Feature Extraction generates one visual shape result file (.shp) for each tiff image file (.tif). For multiplex Agilent microarrays (more than one microarray on a single glass slide), a single .shp file is for the entire tiff image and covers all the arrays on the slide.

The shape file is only viewable in Feature Extraction. It provides visual results about the extracted microarray image. You can view extraction results such as feature cookies, gene names, raw signals, log ratios, outliers, and pixels used to quantify features (available for high-resolution images).

Load the visual results file (.shp) from Project Explorer

1 Right-click a processed extraction set.

2 Click **View Visual Result**.

The image is loaded, along with its shape file into the Image workspace. If no shape file is present, the menu item is grayed out.

Load the visual results file (.shp) from the Image workspace

1 Click **Open Image**, select the .tif file, and click **Open**.

You can also open the image from Project Explorer (right-click the image name and select **View Visual Result)** or the Extraction Set Configuration tab sheet (double-click the Scan File Name).

2 Click **Feature Extraction > Load Visual Result**.

3 Select the .shp file associated with the image, and click **Open**.

Work with the visual results

By default, the when you load a visual results file, Feature Extraction shows the image in View Outliers Only mode, displaying information about outlier features only. To view results for non-outlier features, see "View results on low-resolution images:" on page 118, and "View results on high-resolution images (2- by 3- micron or 20-bit):" on page 118.
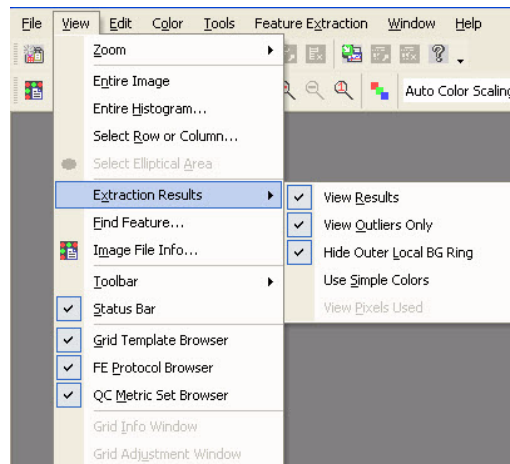
Figure 26   View Extraction Results—Default Settings

View results on low-resolution images:

1 After .shp file is loaded to the image, zoom in until you see enough of the spots to help you evaluate the results.

2 Click the cropping mode icon to turn off crop mode. ⊭

The cursor turns from the cropping cursor to the arrow cursor.

3 Click **View > Extraction Results**, and clear the **View Outlier Only** check box.

4 On the image, pass cursor to feature of interest and hold for a few seconds.

A description of the feature appears.

To find a specific feature, use Ctrl-F. Type the Feature number of the specific feature you want to find.
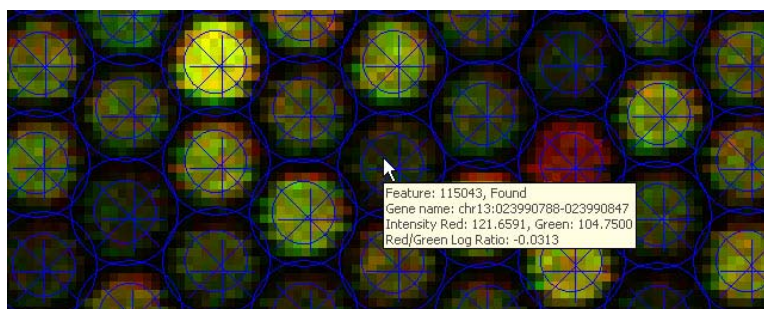
Figure 27   Visual results for extracted microarray image

View results on high-resolution images (2- by 3- micron or 20-bit):

After you first load the image, you, in fact, are viewing a preview image. You can only view the original high-fidelity image if you crop the size of the image to one-tenth or less of the original size.

1 After .shp file is loaded to the image, zoom in until you see enough of the spots to help you identify the region of interest.

2 Click the left mouse button at the point where you want to start forming a rectangle for cropping.

3 With the crop cursor, draw a rectangle equal to or less than one-tenth the size of the image.

Note that the crop cursor is black if the size is within one-tenth (200% to 1000%) of the original size, but it turns red if you are drawing outside the high fidelity range.

4 Then release the mouse button.

Note the status bar at the bottom of the window. It should now read "**High Fidelity**".

5 Click **View > Extraction Results**, and clear the check box **View Outlier Only**.

6 On the image, pass cursor to feature of interest and hold for a few seconds.

A description of the feature appears like in Figure 27 on page 118.

View Pixels Used

This option is available for low-resolution and high-resolution images that are in "High Fidelity" mode (i.e. when the image is zoomed in to one-tenth of its original size).

The **View Pixels Used** option displays the pixels used to quantify the feature signal within the cookie area. Pixels that are determined to be outliers are shown in black.

1 Complete steps 1-5 from "View results on high-resolution images (2- by 3- micron or 20-bit):" on page 118.

2 Under **View > Extraction Results**, select **View Pixels Used**.
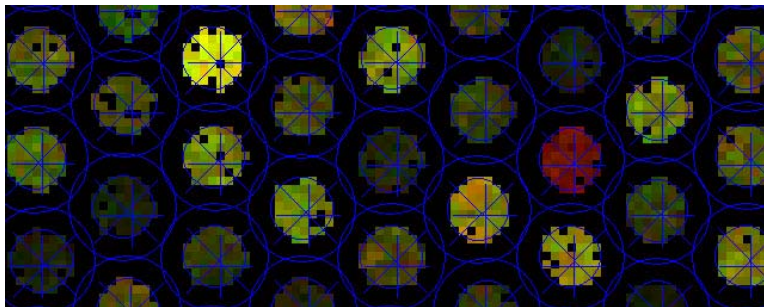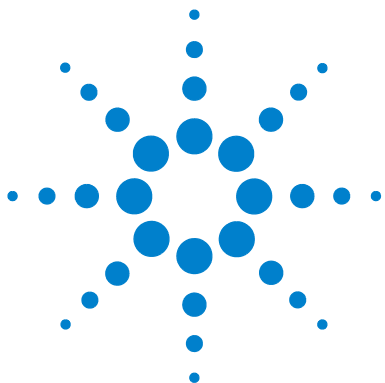
Figure 28 View Pixels Used results on a high-resolution image in high-fidelity mode

3
Creating Grid Files

Agilent Feature Extraction software uses grid templates and grid files to determine where spots are located on a microarray before extraction.

This chapter shows you how to troubleshoot failed Agilent image extractions and create grid files for Agilent and non-Agilent images.

Each section of this chapter describes the actions you take to properly position a main grid, subgrids, and ultimately the spot centroids. The section also gives you background information to help you position these grids and spot centroids.

Agilent Technologies

About grid templates and grid files

A grid template contains spacing information for the specified format as well as a unique set of probes and annotations for the microarray design that it matches.

Feature Extraction automates the process of locating microarray spots through the use of grid templates or grid files, which contain spacing information required to place grids for specific Agilent microarray designs. Grid templates are derived from array design files of the same name that reside in the shared Feature Extraction/Agilent Genomic Workbench database and are displayed in the Feature Extraction Grid Template Browser. When you import, add, or update a grid template, you actually import, add, or update the entire array design file.

A grid file is similar to a grid template, except it contains information specific for a single scan of a given microarray slide.

If gridding fails for a particular Agilent microarray, you may have to create an Agilent grid file to use for the extraction.Agilent grid files are saved using the image name followed by _grid.csv. If you specify a grid file in a project for extraction of an Agilent image, you must also specify the grid template to be used for the rest of the extraction steps.

For non-Agilent microarrays in a project, you must create an Agilent grid file for the specific non-Agilent microarray you intend to extract. A non-Agilent grid file is stored as a .CSV (comma separated value) file and is not saved in the database. When you save a non-Agilent grid file, two .CSV files are actually created that together define the grid. They are named using the image name followed by _grid.csv or _feat.csv. Both files must be present in order to define the grid.

You cannot, nor do you need to, adjust spot centroids for Agilent images because Agilent array printing is accurate once an initial grid is found.

When you create a non-Agilent grid file, you must locate the spot *centroids*, or geometric centers, on the microarrays. To locate spot centroids you interactively position a main grid and subgrids on the microarray. After the subgrids are well-placed, you calculate spot size and centroids.

The software algorithm should locate most, if not all, of the spots where you want them. Any that are incorrect can be moved using a "Spot navigator" (see "Locating spot centroids

manually" on page 162). This ensures that the Feature Extraction software identifies the correct feature and local background.

When to use manual gridding

Table 3   Uses of manual gridding

Image Type	Reason to use manual gridding	See
Agilent Image	Look at how the grid file or template has placed the grid	"Analyzing the initial grid for an Agilent image" on page 125
Agilent Image	If a gridding error appears in the Summary Report after Feature Extraction	"If automatic gridding for an Agilent image fails" on page 127
Non-Agilent Image	Create a grid file to be used with the image for Feature Extraction	"Creating and using a grid file for non-Agilent images overview" on page 131
Agilent or non-Agilent image	Grid is marked as "unplaced" in Extraction Set	"Optimizing grid placement using the help wizard" on page 146
Non-Agilent Image	Evaluation of grid placement	"Starting grid mode for a non-Agilent image" on page 134
non-Agilent image	Spot spacing or location of centroids is not correct	"Calculating spot size and spot centroids" on page 161 "Locating spot centroids manually" on page 162
Agilent or non-Agilent image	Grid placement is not correct after automatic placement	"Manual grid adjustments" on page 155
Agilent (rarely) or non-Agilent image	Grid does not fit the spots on the grid (spacing is incorrect in the Geometry)	"Refitting a grid" on page 169

Analyzing the initial grid for an Agilent image

When you add an Agilent image to a project, the software assigns a grid template, based on the barcode (AMADID) and a protocol. The protocol uses the grid template to automatically find the spots. Normally, you will not need to do any grid adjustments for an Agilent image, however if you want to display the placement of the grid, or if the grid is labeled as "unplaced" you can open the extraction in grid mode and make adjustments.

To open the current extraction in grid mode

For Agilent images, the grid template associated with the image is automatically assigned to the extraction set when the grid template is available in the Grid Template Browser, or if the user has configured eArray to automatically download templates.

1 Open a project file, or add an extraction to an existing project file.

2 If no grid is open, drag and drop a grid template from the Grid Template Browser, or right click on the grid icon and click **Select Grid File** to select an existing grid file.

3 Select a protocol.

You must select a protocol that matches the image for grid mode. If not, the grid and spot placement may not be correct.

4 In the Project Explorer, right-click on the extraction set and select **Open Current Extraction in Grid Mode**.

You can toggle back and forth from grid mode to the project explorer from the Window menu on the toolbar.

The software attempts to place the grid and the image opens in grid mode. In grid mode, you can view the grid placement and make adjustments if necessary.

See "Optimizing grid placement using the help wizard" on page 146.

Main Grid View

Grid Adjustment pane   **Help Wizard**   **Main Grid Corner View**
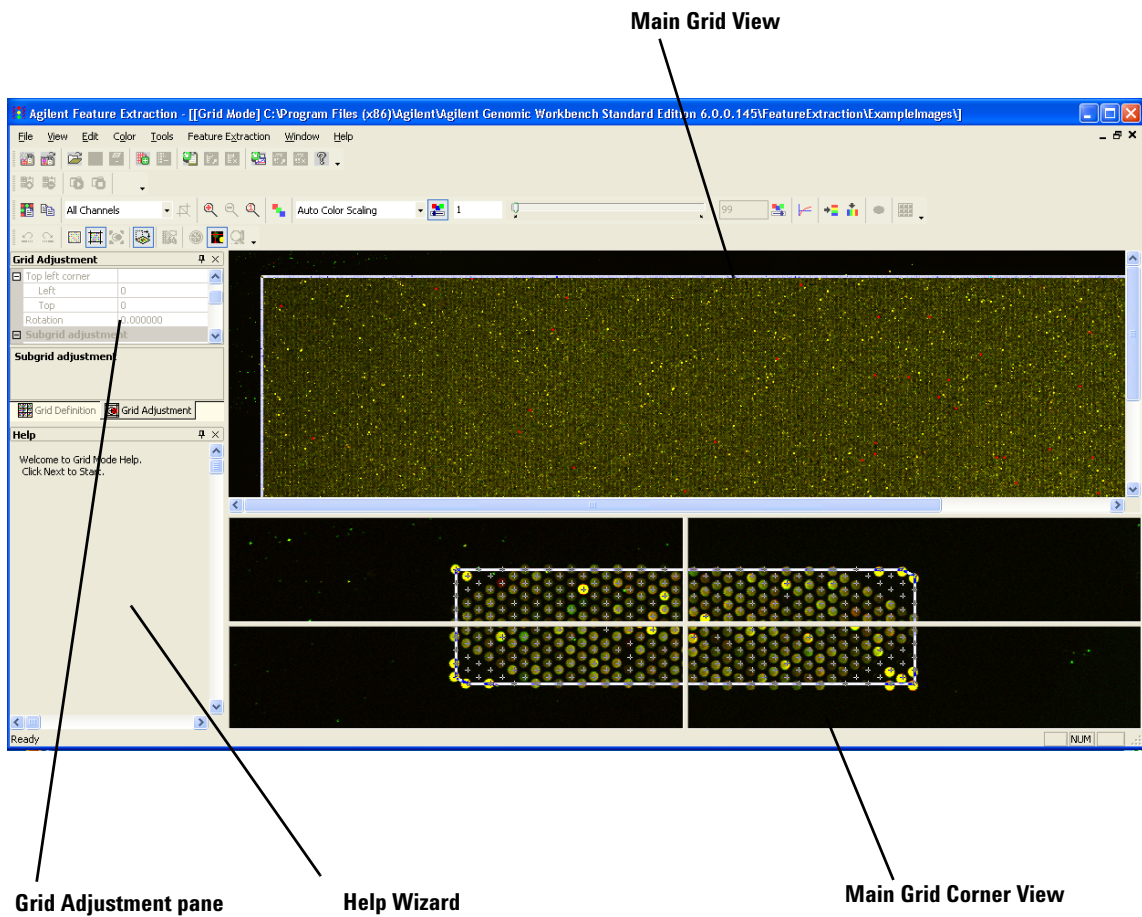
Figure 29   Extraction opened in grid mode

If automatic gridding for an Agilent image fails

If the gridding fails during feature extraction, you will see a red error message in the Summary Report that indicates why the gridding failed. See "To display the Project Run Summary Report" on page 112 for details on summary reports. If gridding fails, follow the steps below to adjust the grid and rerun an extraction set.

Step 1. Create a new project for failed extraction sets

Step 2. Access grid mode from a project extraction set

Step 3. Position grids and save grid file

Step 4. Set up and rerun Feature Extraction

Step 5. Adjusting rotation and skew of subgrids

Step 1. Create a new project for failed extraction sets

When you attempt to close a project or exit the Feature Extraction software after an extraction where one or more extractions failed due to a gridding error, the software will ask if you want to create a new project containing the failed extraction sets.

Create new project with with extraction(s) that were failed due to gridding error

⚠ You are asking to create a new project with extraction(s) that were failed due to gridding error?

FE will close your current project and will create grid files for all the extraction sets in new project.
Note: This operation may take long time.
Do you want to continue?

[Yes]   [No]
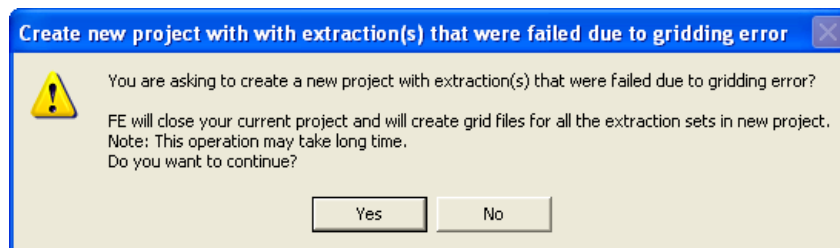
Figure 30   Failed extractions due to gridding error

If you click **No**, the project or Feature Extraction software will close.

If you click **Yes**, the software will

• Create a grid file for all the failed extractions (due to gridding error)

• Create a project for all failed extractions (due to gridding error)

• Created grids are automatically assigned to their respective extraction sets and their status is changed to "unplaced".

The name for the newly created project is "Parent project name_2". Every time a new project from leftover sets is created, the software appends _2 to the parent name.

You must save the new project before you can extract or exit the project.

An *unplaced* grid is one whose x- and y- coordinates, skew, and rotation are equal to zero.

When you run the project containing the failed extraction sets, Feature Extraction will check the grid placement status of the first extraction set. If the grid is "unplaced" a message will appear asking you if you want to open grid mode. Click **Yes** to open the extraction in grid mode where you can place and save the grid. If you click **No**, the software will check the next extraction set in the project until all extraction sets have been checked.

Step 1b. Manually create a new project for failed extraction sets

If a run fails for any reason, you can create a project containing the failed or not yet processed extraction sets left over from the previous project run. The following requirements must be met:

• Project must be a Standard FE project

• Project must still be in the Run mode

• Project has just stopped extracting and contains failed extraction set(s) or extraction set(s) not yet run.

1 Select **Project > Create Project for Leftover Set(s)**.

A message appears asking if you want to switch back to Config mode and continue.

2 Click **Yes**.

Feature Extraction creates a new project with all unfinished extraction sets from the current project. Feature Extraction also closes the current project and starts the new project in Config mode.

The name for the newly created project is "Parent project name_2". Every time a new project from leftover sets is created, the software appends _2 to the parent name.

You must save the new project before you can extract or exit the project. To proceed, open the extraction set in grid mode by following Step 2, below.

Step 2. Access grid mode from a project extraction set

This is the quickest way to access grid mode after automatic grid placement has failed. It is the only way to access grid mode for Agilent images.

After gridding, the extraction set *consists of the image file, the grid template and grid file, and the protocol.*

In the Project Explorer on the left side of the project window, right-click on any object in the extraction set (extraction set name, image, grid template or file, protocol).

3 Select **Open Current Extraction in Grid Mode**.

The image file for the extraction set appears in grid mode, ready for positioning of the main grid.

Also, the grid file appears under the original grid template in the extraction set.

Step 3. Position grids and save grid file

1 Open the extraction in grid mode.

2 Follow the help wizard steps to:

   a Position the main grid.

The spot size and spot centroid options in the Help Wizard are used for non-Agilent images only.

   b Position subgrids, if necessary.

   See "Optimizing grid placement using the help wizard" on page 146.

The grid is labeled as "unplaced" in the Project Explorer until the grid is positioned and grid mode is closed.

3 Save the grid file.

   See "Saving a grid file" on page 160.

Step 4. Set up and rerun Feature Extraction

1 Close the grid mode window.

2 Save the project.

3 Click the **Project Run mode On/Off** icon to start the extraction.

Creating and using a grid file for non-Agilent images overview

Follow these instructions to create and use a grid file for non-Agilent images. You must create a grid file for non-Agilent images to run Feature Extraction.

Step 1. Start grid mode from the non-Agilent image file.

Step 2. Position grids, locate spots and save grid file.

Step 3. Extract the image file with its new grid file.

Step 1. Start grid mode from the non-Agilent image file

1 Open the non-Agilent image file.

See "To open an image file" on page 134.

Grid mode cannot be accessed from an open Agilent image. For Agilent images, you must access grid mode from within an extraction set. See "Step 2. Access grid mode from a project extraction set" on page 129.

2 Open grid mode and select the gene list type and protocol to use.

See "To open grid mode for a non-Agilent image" on page 137.

3 Click **OK**.

4 In the Geometry Information dialog box, click **Auto Fit**.

The image file for the extraction set appears in grid mode, ready for positioning of the main window. See "Geometry information" on page 143.

Step 2. Position grids, locate spots and save grid file

If you are using a non-Agilent microarray, the decision to adjust spots, calculate spot sizes and centroids, or position spots manually is based on visual inspection of how the grid is fitted.

The spot size and spot centroid options are used for non-Agilent images only.

1 Follow the help wizard steps in the lower left corner of the grid mode window to:

  a Determine nominal spot and subgrid spacing manually. (Optional and only for third-party arrays.)

  b Determine nominal subgrid spacing. (Optional and for third-party arrays with subgrids only.)

  c Position main grid and subgrids, if necessary.

  See "Optimizing grid placement using the help wizard" on page 146.

2 Click the **Adjust Spots** icon to enable spot location adjustment.

3 Click the **Calculate spot size and centroids** icon and calculate the spot sizes and centroids.

  See "Calculating spot size and spot centroids" on page 161.

4 Visually inspect the spot centroids and if necessary, position spots manually.

  See "Locating spot centroids manually" on page 162.

5 Save the grid file.

  See "Saving a grid file" on page 160.

Step 3. Extract the image file with its new grid file

To test the new grid file, create the project and add the extraction set.

1 Select **Feature Extraction > Create a New FE Project**.

A new Feature Extraction project is created with the image file already added.

2 In the Project Explorer, right-click on the grid icon below the image file, and click **Select Grid File**.

3 In the Select Grid file for Extraction Set dialog box, select your saved grid file, and then click **Open**.

4 From the FE Protocol Browser, drag the protocol for the image type to the extraction set.

5 Save the project and run Feature Extraction to see if the grid works.

Starting grid mode for a non-Agilent image

You can view and adjust grid placement using grid mode. For non-Agilent images, grid mode can be accessed from either an open image file or from an extraction set that has an associated grid and protocol.

To open an image file

You cannot access grid mode for Agilent images from an open image. See "Step 2. Access grid mode from a project extraction set" on page 129.

You can also open an image file by dragging the file to the Feature Extraction desktop icon.

1 Click on the toolbar **Open image file** button, or click **File > Open > Image**, to display the Open Dialog Box.

2 In the Open Dialog Box, double-click on a .tif file from the scanner, or select a file and click **Open**.

An image of the scanned microarray contained in the file opens in its own window.
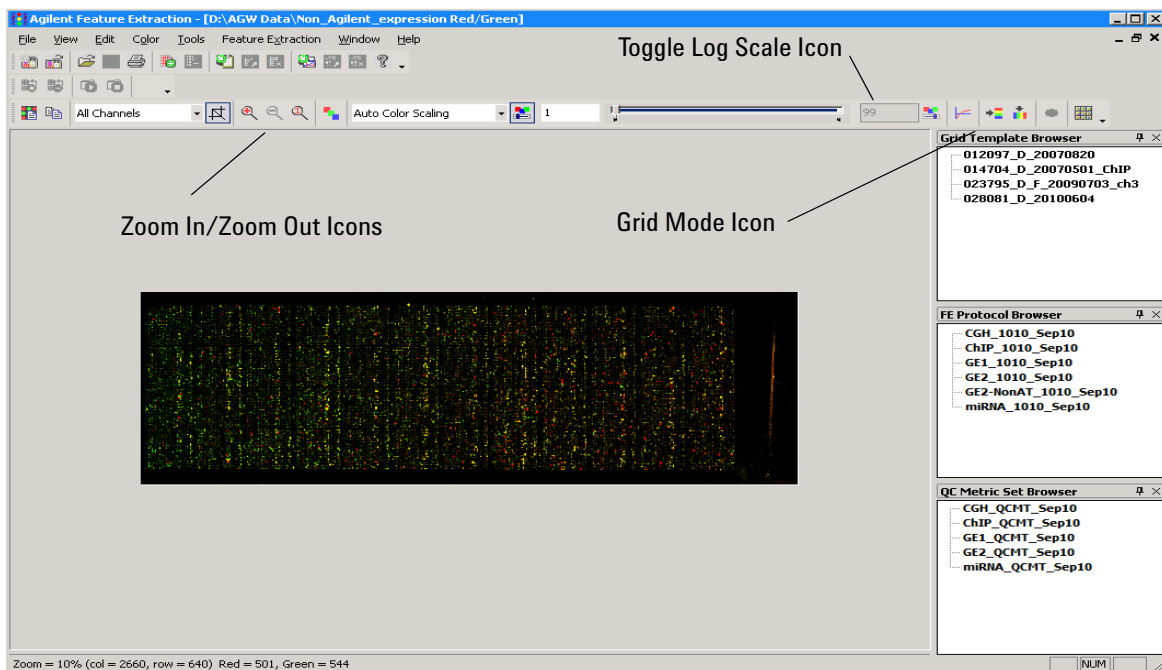
Figure 31   TIFF image for non-Agilent microarray

3 To see the spots more clearly, click the **Toggle Log Scale** icon and click the **Zoom In** icon to increase the image size.
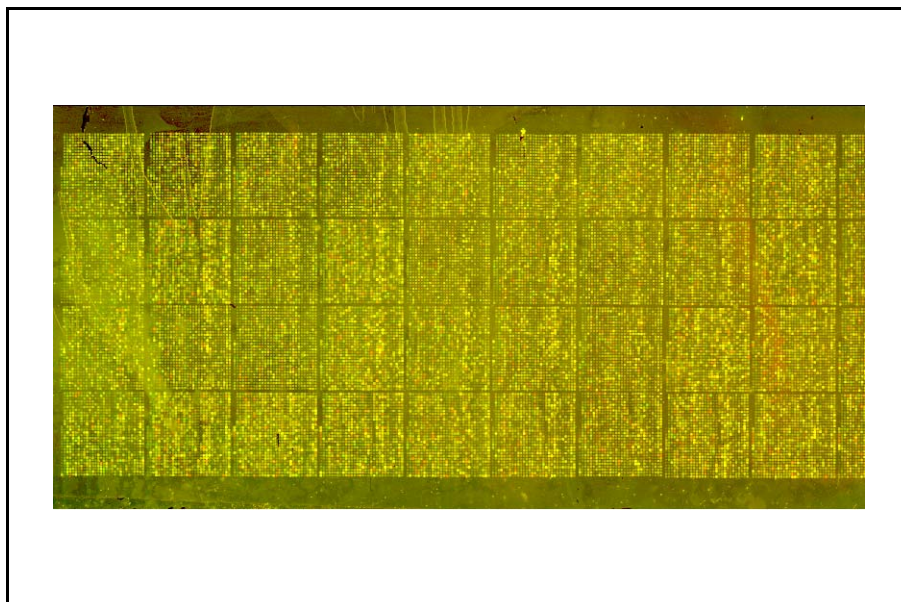
Figure 32   Image view with log scale on and image zoomed

To change the Portrait/Landscape view of the image

The software changes orientation in this way because the Agilent scanner scans the long side of the microarray through the glass. This is an orientation that is flipped and rotated compared with other scanners.

You must change the orientation of any opened image if the gene list file that you intend to use to provide grid information organizes its data with the opposite orientation. You typically do not have to do this for Agilent images, but you usually have to do this for non-Agilent images.

1 Select **Tools > Flip Upper Left to Lower Right (Landscape/Portrait)**.

   The software takes the upper left corner of the image and flips it to the lower right no matter if you change the orientation from Portrait to Landscape or vice-versa.

2 Select **File > Save modified image** to save the image.

To open grid mode for a non-Agilent image

1 Open a non-Agilent image file.

2 Click the **Grid** button on the image toolbar.

   The Create/Edit Grid dialog box opens.
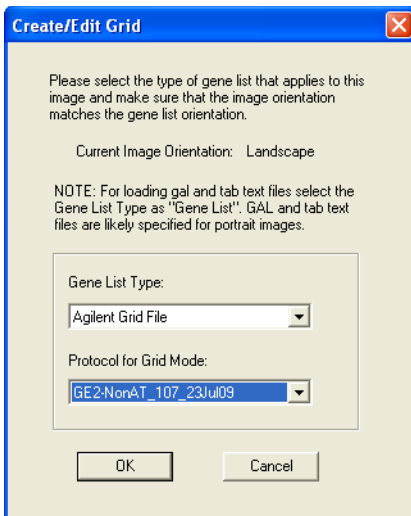
Figure 33   Create/Edit Grid dialog box

3 Select the Gene List Type to let you position the grid.

If you select a file whose data orientation is different from that of the image, the Agilent software will not be able to set the grid properly. Cancel out of this dialog box and change the orientation if you have to.

Agilent Grid File (*_grid.csv)   This is a grid file that has been saved from the previous creation of a grid on an image.

If you want to edit a grid or change the location of the spot centroids for an image, select the grid file previously saved for that image.

If you have never set up an initial grid and the image resembles one whose grid you have set up before, select that grid file from the already existing grid files.

Gene List   Select this if the image that you opened has an associated GAL or tab text file.

The layout information and feature annotations must be in tab-delimited text format and must contain at least these columns: Grid index (or SubGridRow/SubGridCol), SpotRow, SpotCol and GeneName or Probename.

The tab text file can be a GAL file, a results file or an Excel file exported as a tab-delimited text file.

Control Type can also be in the tab text file. See the *Feature Extraction 10.10 Reference Guide* to see the definitions of the control types that can appear in the tab text file.

No Gene List   If you have no geometric information associated with the microarray, select this option. You can also select this option if another file you have used does not result in a workable grid. If you select this option, the Feature Extraction output file does not contain any feature annotations.

4 Select a protocol.

For 2 color Gene Expression, the default GE2-NonAT_1010_Jun10 protocol provided with the software can be used for non-Agilent images.

Select a protocol to help with grid placement and spot finding. Grid placement and spot finding algorithms differ between protocols. You must select a protocol that matches the image for grid mode. If not, the grid and spot placement may not be correct.

5 Click **OK**.

What happens next will depend on what you have selected for the gene list type:

If you select Agilent Grid File as the gene list type

A Browser appears that lets you select the grid file. The grid file must already exist for the image.

• Select a grid file, and click **Open**.

   The Main Grid View appears with the grid approximately positioned.

If you select Gene list and then select a gal file as the gene list type

The Geometry Information dialog box appears for the gal file grid.

1 Change the default values if you need to.

2 Click **Auto Fit** or **Manual Fit**.

   For a description of all the fields and buttons in the Geometry Information dialog box, see "Geometry information" on page 143.

   The Main Grid View for this image appears.

If you select Gene list and then select a tab text file as the gene list type

The Tab Text Grid File Preview window appears.
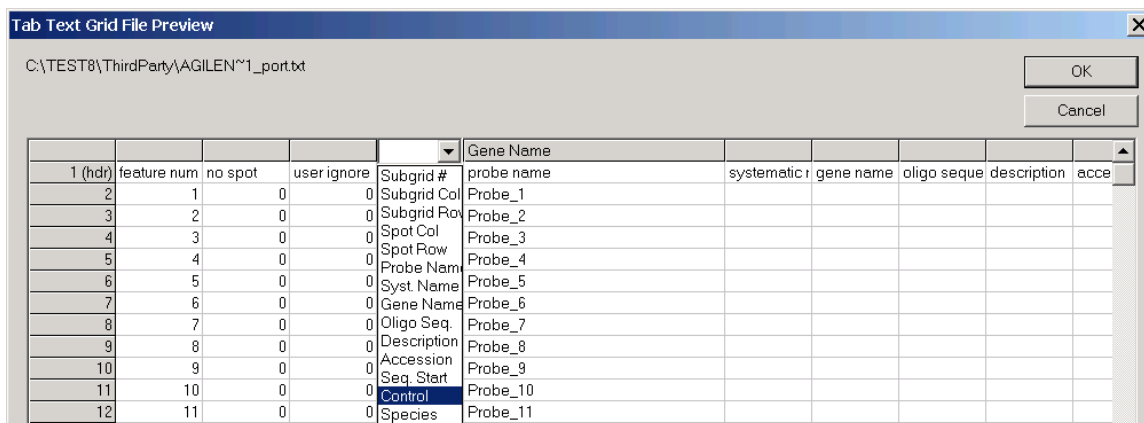
Figure 34   Tab Text Grid File Preview window with header selected

1 Click each of the column headers that correspond to the following information fields:

- Subgrid Col
- Subgrid Row
- Spot Col
- Spot Row
- Probe Name

2 Select the appropriate name from the list.

You must change the names of the columns listed above in order for gridding to work with a tab text file. You can also change other column names, such as Gene Name or Control.
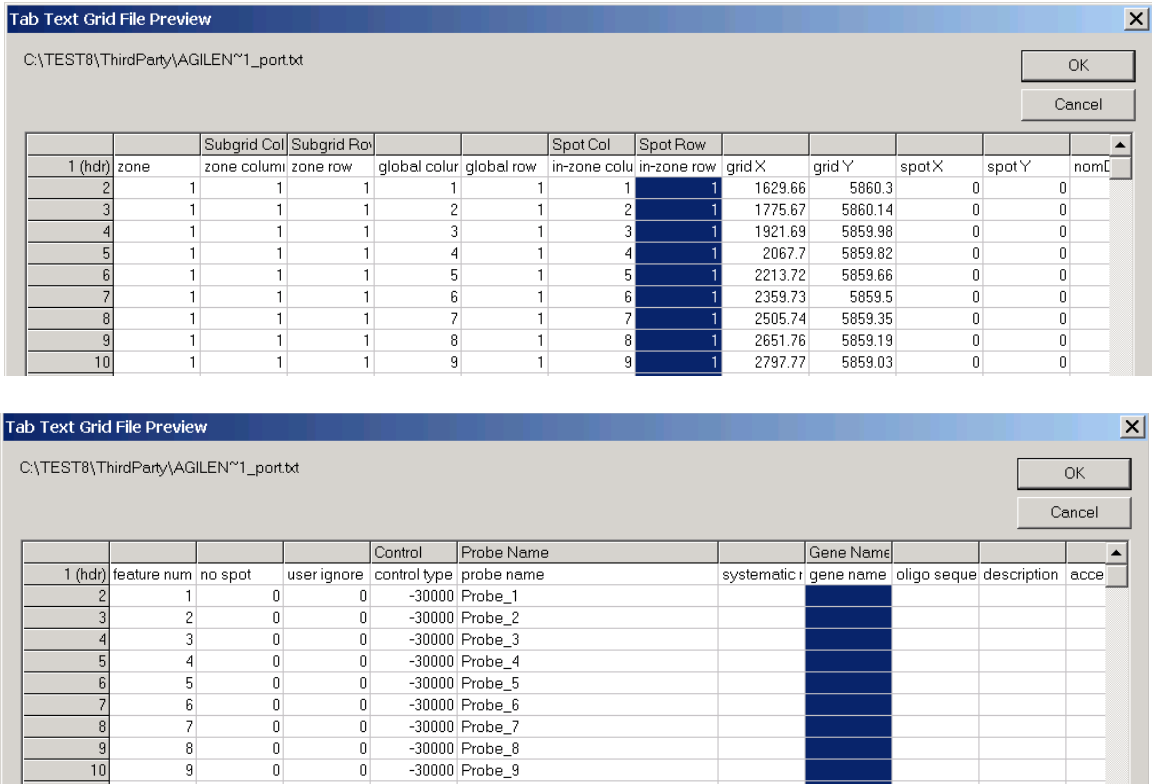
Figure 35 Tab Text Grid File Preview window with headers selected

3 Click **OK**.

The Geometry Information dialog box appears.

4 Click **Auto Fit** or **Manual Fit**.

For a description of all the fields and buttons in the Geometry Information dialog box, see "Viewing and changing grid geometry" on page 144.

The Main Grid View for the non-Agilent image appears.

If you select No Gene List

The Geometry Information dialog box appears.

1 Change the default values if you need to.

2 Click **Auto Fit** or **Manual Fit**.

For a description of all the fields and buttons in the Geometry Information dialog box, see "Viewing and changing grid geometry" on page 144.

The Main Grid View for the non-Agilent image appears.

Geometry information

This dialog box appears when you click OK from the Create/Edit Grid dialog box.
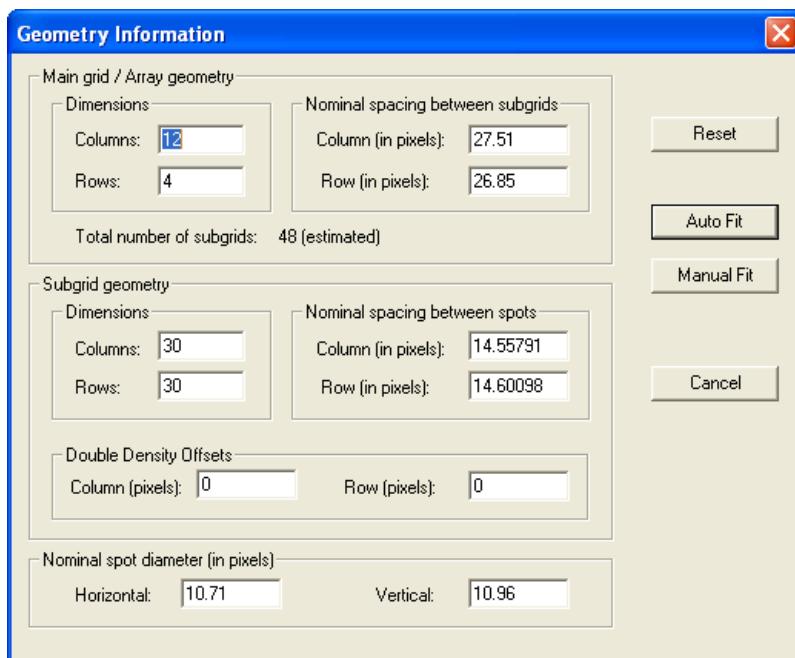
If Gene List or No Gene List are selected as the Gene List Type in the Create/Edit Grid dialog box, the Feature Extraction software attempts to discover the grid and will populate the Geometry Information fields with the information from that attempt.

For Gene List, if you select a .gal file, the parameters from the .gal file is displayed in the Geometry Information dialog box.

For details on all of the fields and buttons of the Geometry Information dialog box, see "Viewing and changing grid geometry" on page 144.

Viewing and changing grid geometry

The following dialog box appears when you click OK from the Create/Edit Grid dialog box when entering grid mode from a non-Agilent image file (see "Starting grid mode for a non-Agilent image" on page 134), or when you Refit a grid (see "Refitting a grid" on page 169).

Figure 36   Geometry Information dialog box

Main grid/Array geometry   For the main grid, enter dimensions in the form of number of columns and rows, or enter the distance between subgrids in pixels for both columns and rows.

Subgrid geometry   For subgrids, enter dimensions in the form of number of columns and rows, or enter the distance between spots in pixels for both columns and rows.

Double Density Offsets   The double density offset is applied when the array being gridded contains features consisting of two interlaced rectangular grids. The spacing of these rectangular grids is specified in the Double Density row and column offsets.

Nominal spot diameter   Because spots may not be circular, enter the horizontal and vertical dimensions in pixels.

Reset   Click this to reset the displayed parameters to their original values.

Auto Fit   The software uses the entries for Geometry Information as a starting point for placing the grid, then adjusts the subgrids for the best fit and identifies the spot centroids on the image. Choose this unless you know the automatic grid fit is not correct.

Manual Fit   The software places the grid on the image, usually in the upper left-hand corner. You then align the grid with the corners of the image. Based on this grid fit, the software identifies the spot centroids. Choose this if you have tried Auto Fit and you need to fit the grid manually.

Cancel   Click this to close the dialog box and return to the image display.

Table 4   Geometry information that you can change on the Geometry Information (GI) dialog box[*]

File Type	Initial GI dialog box displays?	Change main grid dimensions?	Change subgrid dimensions?	Change spot spacing?	Change spot size?
Agilent Grid	No[†]	No	No	Yes	No
GAL Format	Yes	Yes	No	Yes	Yes
Tab Text	Yes	Yes	No	Yes	Yes
No gene list	Yes	Yes	Yes	Yes	Yes

*   You can change the nominal spot and subgrid spacing in grid mode.

†   The Geometry Information dialog box does not appear when you select Agilent Grid File as the gene list type. The columns for the Agilent Grid file type apply to the "refit" Geometry Information dialog box, which does appear when you attempt to refit an Agilent grid (see "Refitting a grid" on page 169).

Optimizing grid placement using the help wizard

Although the Help Wizard is available for both Agilent and non-Agilent arrays, some steps apply only to non-Agilent (third-party) arrays.

Grid mode provides a Help Wizard to step you through the process of optimizing the placement of the grids. To view the help steps, in the Help pane on the lower left of the grid mode window, click **Next**. To go back to the previous step, click **Previous**. The help wizard provides instructions for the following tasks:

Step 1. Get nominal spot spacing manually (for non-Agilent arrays only)

Step 2. Get nominal subgrid spacing manually (for non-Agilent arrays only)

Step 3. Adjust the position of the main grid

Step 4. Adjust the subgrid

Step 5. Adjusting rotation and skew of subgrids

Step 6. Save grid file

To zoom in or out of the views

Before you optimize the grid placement, it may be necessary to zoom the image in order to see the spots more easily. There are two ways to zoom the image:

See "To change the magnification (zooming)" on page 279 of this guide for more details on zooming.

• From the Feature Extraction menu bar, select **View > Zoom** and choose a % zoom from 10% to 1000%. A 10% zoom is defined and you can select a zoom level in 10% increments. You can also change the default zoom settings. See "Changing default settings for Grid Editing" on page 171 for details.

• On the image toolbar, click one of the zoom icons.

Click to zoom in. Click to zoom out. Click to zoom to 100%. Main Grid View

Image Toolbar

Grid Adjustment pane Help Wizard Corner View

Figure 37   Grid mode view

To undo or redo grid position movements

- Click the **Undo** button, ⤺, to undo an action.
- Click the **Redo** button, ⤼, to redo an action.

Step 1. Get nominal spot spacing manually

This step is optional, and is only used for third-party arrays. Perform this step if spacing information for spots are unknown.

1 Make sure that the color log scale ⊬ has been turned on to view the corner spots on the main grid more easily.

2 On the gridding toolbar, click the **View and Measure Image** icon ▥ .

3 If necessary, zoom the image. See "To zoom in or out of the views" on page 146.

4 On this image view (which is appended with [CROPPED] in the image name), click on the center of the top left spot and drag the mouse horizontally to the center of the spot in the next column. (Do not release the mouse button.) Read and note the column distance from the status bar. This is the left-hand number shown in parentheses; for example, (16x0). (You will need this value later.)

5 On the image view, click on the center of the top left spot and drag the mouse vertically to the spot center in the first column of the second row. (Do not release the mouse button.) Read and note the row distance from the status bar. This is the right-hand number shown in parentheses; for example, (0x17). (You will need this value later.)

6 Close the cropped image view.

7 In the grid mode view, click the **Grid Definition** tab.

8 In the Grid Definition panel, double-click on the space next to **Col** under Nominal Spot spacing, and enter the column distance value from step 4.

9 In the Grid Definition panel, double-click on the space next to **Row** under Nominal Spot spacing, and enter the row distance value from step 5.
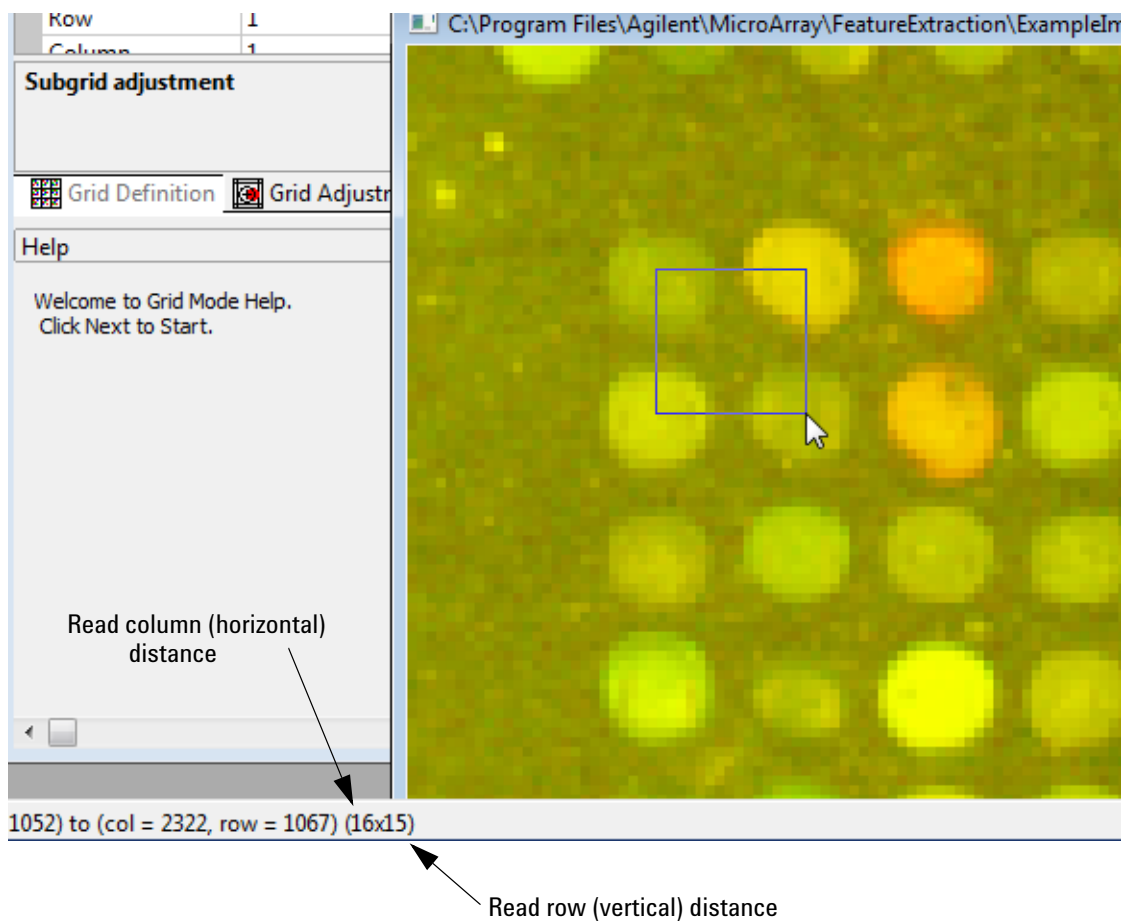
Figure 38 Reading column or row distance from image view

Step 2. Get nominal subgrid spacing manually

This step is optional and only for third-party arrays. Perform this step if spacing for the subgrids is unknown.

1 Make sure that the color log scale ⊬ has been turned on to view the corner spots on the main grid more easily.

2 On the gridding toolbar, click the **View and Measure Image** icon ⊡ .

3 If necessary, zoom the image. See "To zoom in or out of the views" on page 146.

4 On the newly-opened image, click on the center of the top right spot for the first subgrid and drag the mouse horizontally to the center of the first spot in the adjacent subgrid. (Do not release the mouse button.) Read and note the column distance from the status bar. This is the left-hand number shown in parentheses; for example, (43x0). (You will need this value later.)

5 On the opened image view, click on the center of the bottom left spot of the first subgrid and drag the mouse vertically to the center of the top left spot of the subgrid below. (Do not release the mouse button.) Read and note the row distance from the status bar. This is the right-hand number shown in parentheses; for example, (0x42). (You will need this value later.)

6 Close the opened image view.

7 In the grid mode view, click the **Grid Definition** tab.

8 In the Grid Definition panel, double-click on the space next to **Col** under Nominal Subgrid spacing, and enter the column value from step 4.

9 In the Grid Definition panel, double-click on the space next to **Row** under Nominal Subgrid spacing, and enter the row value from step 5.

Step 3. Adjust the position of the main grid

1 Make sure that the color log scale ⧉ has been turned on to view the corner spots on the main grid more easily.

2 On the toolbar, click the **Adjust Main Grid** icon ⧉ .

3 Move the mouse into the upper Main Grid View until the "move" cursor appears. ⧉

4 Click and drag the "move" cursor so that the top left corner of the grid is positioned on the top left spot of the microarray image.

5 To do fine adjustment of the main grid, click on the top left spot in the top left pane of the lower Corner View pane.
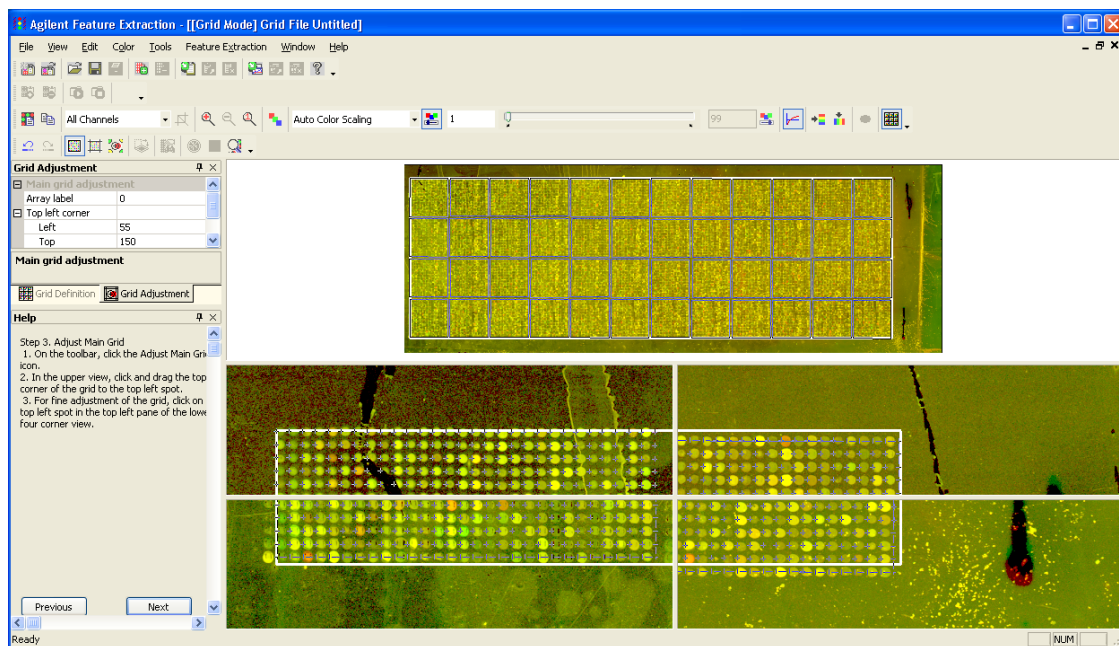
Note that the thick line represents the main grid and the thin line represents the subgrids.

Figure 39　Adjust main grid view

Step 4. Adjust the subgrid

For a microarray with only one grid, if the main grid is well-positioned, there is no reason to move the subgrid. This is the case for most Agilent microarrays.

When you position subgrids you are, in effect, positioning nominal spot centroids (+). These nominal spot centroids are still subject to adjustment by the spot finding algorithm in Feature Extraction. You can position the subgrids such that the nominal spot centroids are just inside the spots. These positions are good enough for the spot finding algorithm to calculate more accurate spot centroids.

1 On the toolbar, click the **Adjust Subgrid** icon.

2 In the Main Grid View, click on a subgrid.

3 In the Main Grid View, click and drag the top left corner of the subgrid to the top left spot of the subgrid.

4 For fine adjustment of the grid, click the **Select Corners and Automatically Fit Grid** icon on the toolbar to turn it off.

5 In lower Corner View, click the center of the top left spot of the selected subgrid.

6 Repeat step 2 through step 5 for the remaining subgrids.
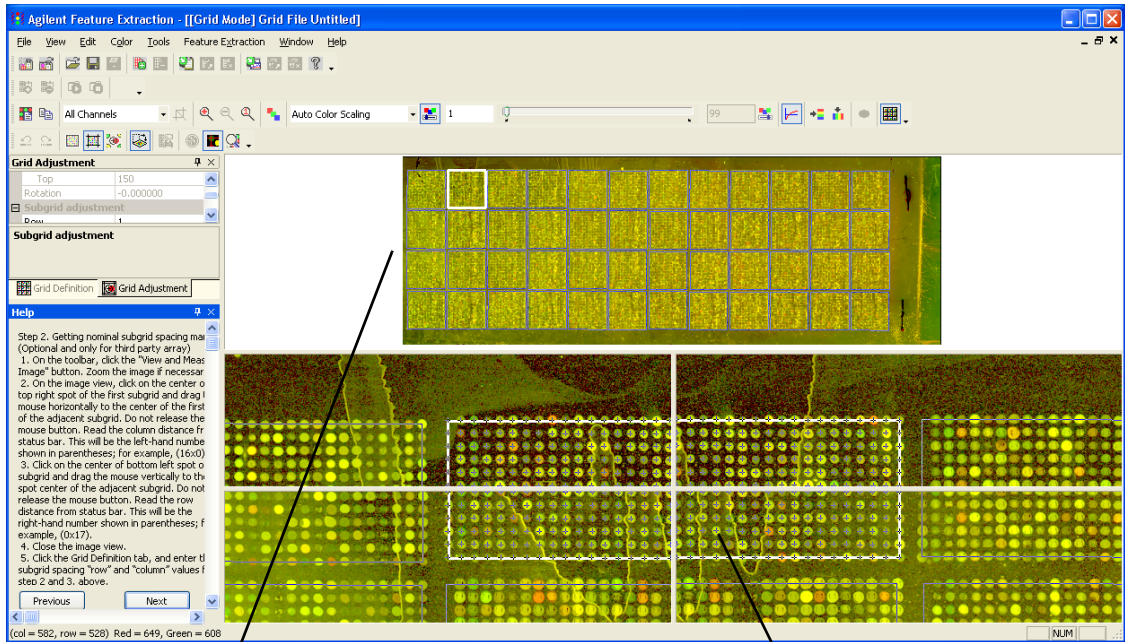
Subgrid Main Image View   Subgrid Corner View

Figure 40   Adjust subgrid view

Step 5. Adjusting rotation and skew of subgrids

*When **Select Corners and Automatically Fit Grid** is enabled, the software will automatically calculate a fit based on three user-selected corner points.*

1 In the Main Grid View, click on a subgrid to select it.

2 On the toolbar, click to select the **Select Corners and Automatically Fit Grid** icon, if it is not on.

3 In the lower view, if the corners of the grid are not near to the corner spots of the image, adjust the grid location by clicking and dragging the grid in the bottom right pane of the lower four corner view.

4 Move the cursor to the top left pane of the lower Corner View. Notice the cursor turns to a red cross.   Click on the top left spot of the subgrid.

5 In the top right pane of the lower Corner View, click on the top right spot (in the first row) of the subgrid.

6 In the bottom left pane of the lower Corner View, click on the lower left spot (in the first column) of the subgrid.

7 Repeat step 3 through step 6 for the remaining subgrids.

Step 6. Save grid file

1 Click the **Save Grid** button on the main toolbar.

2 The grid is saved to a ImageName_grid.csv file where ImageName is the name of the image associated with the grid, and the directory containing the grid file is the same as the directory of the original image. A features file is saved at the same time as a ImageName_feat.csv file.

Manual grid adjustments

For most images, grids are adjusted adequately if you follow the steps provided by the Help Wizard. However, if the placement of the grid is not adequate after performing the steps of the Help Wizard, you can correct the grid placement using the following procedures.
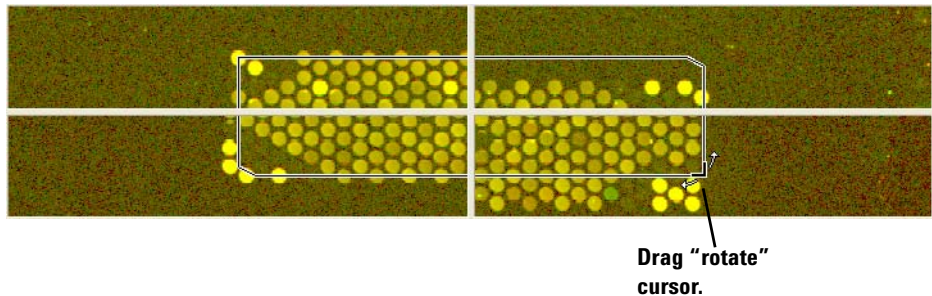
To rotate the position of the main grid

To move or rotate the main grid another way, see "To move or rotate the main grid with Grid Adjustment pane" on page 156.

1 Make sure that the color log scale has been turned on to view the corner spots on the main grid more easily.

2 Click the **Adjust Main Grid** button on the toolbar.

3 Move the mouse to a corner of the main grid in the lower Corner View.

The "rotate" cursor appears.

4 Drag the "rotate" cursor to rotate and optimize the position of the grid on the four corners of the microarray image.

Drag "rotate" cursor.
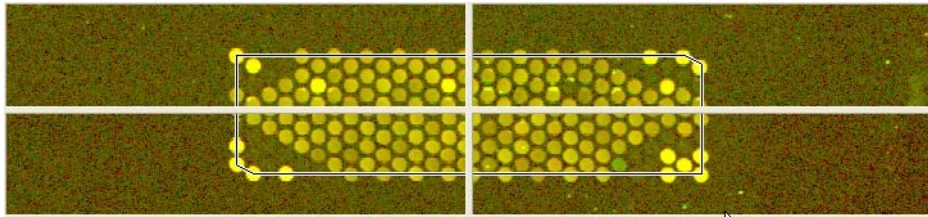
Figure 41   Final position of main grid on microarray

To move or rotate the main grid with Grid Adjustment pane

When the Main Grid View appears, the Grid Adjustment tab is the default selection. You can move or rotate the main grid by entering values for fields in the Grid Adjustment tab.

To learn all the ways that you can move grids and spot centroids, see "Shortcuts to help you work with grids and find spots" on page 172.
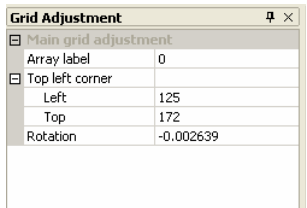
1 Click the **Grid Adjustment** tab, if necessary.

Figure 42   Grid Adjustment pane for Main Grid View

2 Click the **Adjust Main Grid** button on the toolbar.

3 Enter a value for any field that is available to you.

Array Label   This number identifies which of the Agilent microarrays on the slide you are looking at.

Top Left Corner   Enter pixel locations for the top left corner.

Rotation   You can change the number here to rotate this corner of the main grid either in the positive or negative direction.

To rotate the subgrid

If moving the position of the subgrid does not work, try rotating the subgrid.

1 Click to select the **Adjust Subgrid** button on the toolbar.

2 Click to deselect the **Select Corners and Automatically Fit Grid** button on the toolbar (if it is turned on).

3 Click on a subgrid in the upper Main Grid View.

4 In the lower subgrid Corner View, move the mouse to a corner of the subgrid until the "rotate" cursor appears.

5 Drag the "rotate" cursor on the subgrid Corner View to optimize the position of the grid on the four corners of that portion of the microarray image.
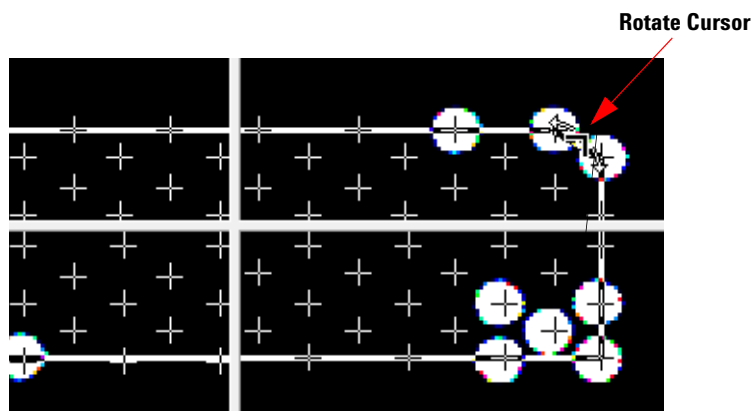
Rotate Cursor

Figure 43   Subgrid with rotate cursor

To skew the subgrid corner

If neither of the above methods work, try skewing the subgrid corner.

1 Click the **Skew Subgrid** button, [icon].

2 Move the mouse to a corner of the subgrid Corner View.

The "skew" cursor appears.

3 Drag the "skew" cursor on the subgrid Corner View to optimize the position of the grid on the four corners of that portion of the microarray image.
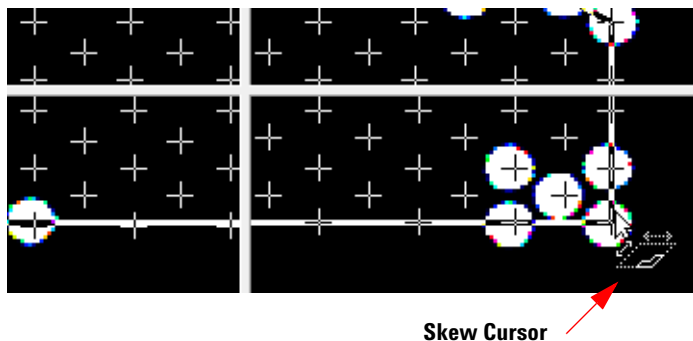
Skew Cursor

Figure 44   Subgrid with skew cursor

To position subgrids with Grid Adjustment pane

When the Adjust Subgrid button is selected [icon], the Grid Adjustment tab is the default selection. You can position subgrids by entering values for fields in the Grid Adjustment tab.

1 Click the **Grid Adjustment** tab, if necessary.

Figure 45   Grid Adjustment pane for subgrids

2 Enter a value for any field that is available to you.

Col and Row Numbers   Click the down arrows for Col and Row to select the column and row numbers for the subgrid you want to position.

Corner of Subgrid   Enter pixel locations for the top left corner of a subgrid.

Rotation   Enter a number to rotate the top left corner of the subgrid.

Skew   Enter a number to skew the top left corner of the subgrid.

Saving a grid file

Whenever you have made manual adjustments to the grid or spacing geometry, you must save the grid file in order to keep the changes you have made. There is only one grid file for any given image.

1 Click the **Save Grid** button on the main toolbar. 🖫

2 The grid is saved to a ImageName_grid.csv file where ImageName is the name of the image associated with the grid, and the directory containing the grid file is the same as the directory of the original image. For non-Agilent images, a features file is saved at the same time, with the name ImageName_feat.csv.

Calculating spot size and spot centroids

This option is available only for non-Agilent images.

This is the same algorithm used for calculating spot size and finding spot centroids by the software during an automatic extraction. For third-party arrays, running this will show you how well the algorithm will find the spots on the array and let you perform corrections, if required (see "Locating spot centroids manually" on page 162).

1 Click the Adjust Spot button to enable spot mode.

2 Click the **Calculate Spot Size and Centroids** button to let you see the difference between the nominal spot centroid, positioned from the grid (+), and the estimated spot centroid based on the spot size (X).
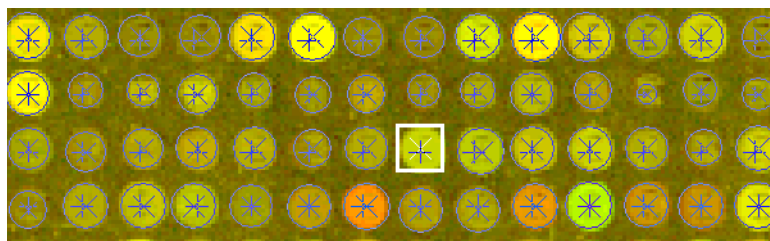
After you have calculated the spot size and spot centroids, you can make adjustments manually. See "Locating spot centroids manually" on page 162. When you move a grid or subgrid after calculating the spot size and spot centroids, the found centroids for the moved grid or subgrid are deleted.

Locating spot centroids manually

If you want to save the manually-placed spots, you must save the grid file.

Once you have calculated the spot sizes and centroids (see "Calculating spot size and spot centroids" on page 161) you can manually adjust those. In this section you learn how to move a nominal spot centroid, placed on a spot by the subgrid. When you move the nominal spot centroid (+), it becomes an estimated spot centroid (X). Once the spot centroid is estimated, the software uses this estimated value for Feature Extraction.

You cannot move spot centroids for Agilent images. This function works only for non-Agilent images.

If a spot centroid has been manually moved, the result called IsManualFlag is set to true. See the *Feature Extraction 10.10 Reference Guide* for the result.

To find a spot to adjust

1 Click the **Adjust Spot** button, 🖼️ ,to bring up the Main Spot View and the Zoomed Spot View.

Adjust Spot button   Spot Navigator button   Preview Spot Centroids button   Main View

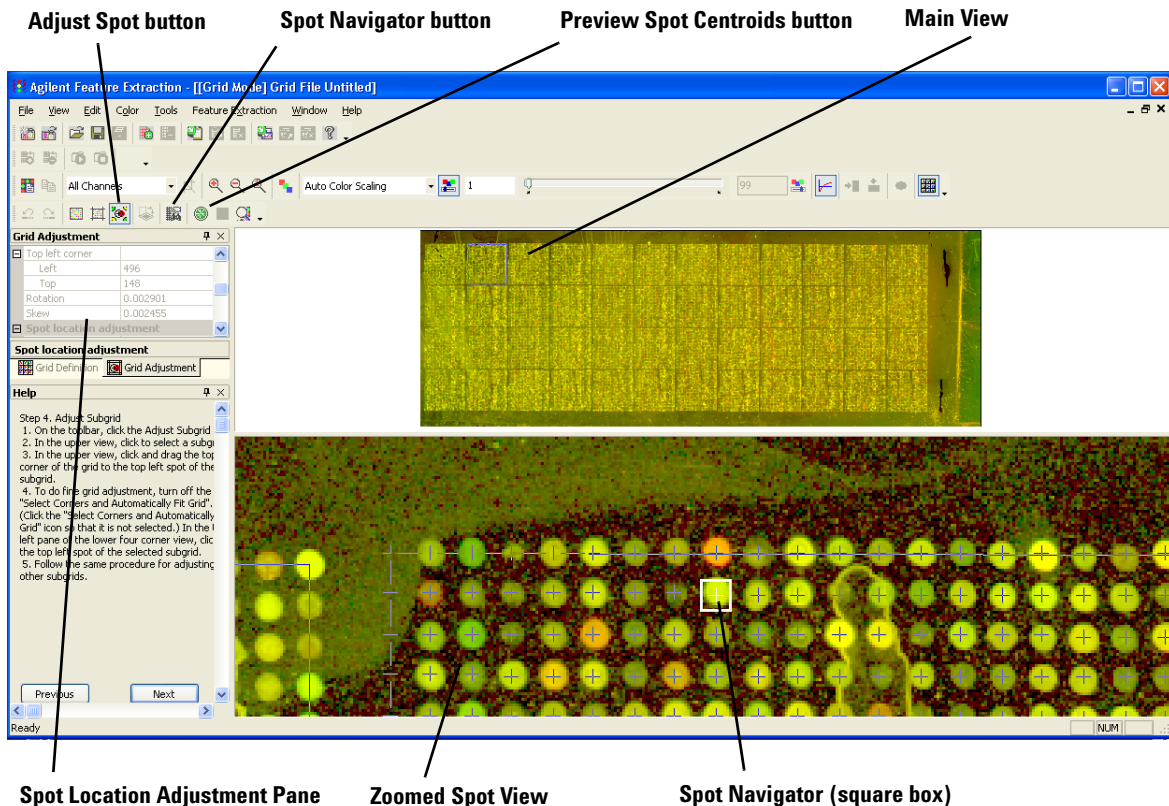Spot Location Adjustment Pane   Zoomed Spot View   Spot Navigator (square box)
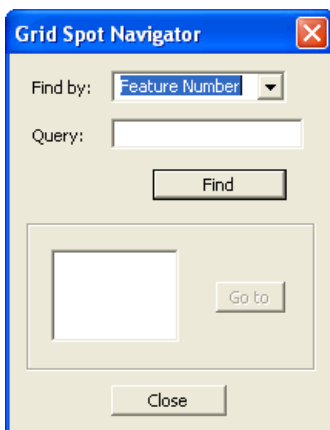
Figure 46   Adjust spot view

The calculated spot centroids appear when you enter Adjust Spot mode.

2 Select **Color > Use Log Color Scale** if this has not already been selected.

3 Click a spot on the Main Spot View or on the Zoomed Spot View.

Or

Click the **Spot Navigator** button, 🖳, to bring up the Grid Spot Navigator dialog box.

4 Click the down arrow in the **Find by** list, and select the number or name of the spot to navigate to.

5 In the **Query** field, type the feature number or probe name that you want to find.

Case is important.

6 Click **Find**.

If only one feature in the microarray matches the query, the software moves the spot square (Spot Navigator) to that feature.

If more than one feature matches, a list of features is displayed in the bottom box.

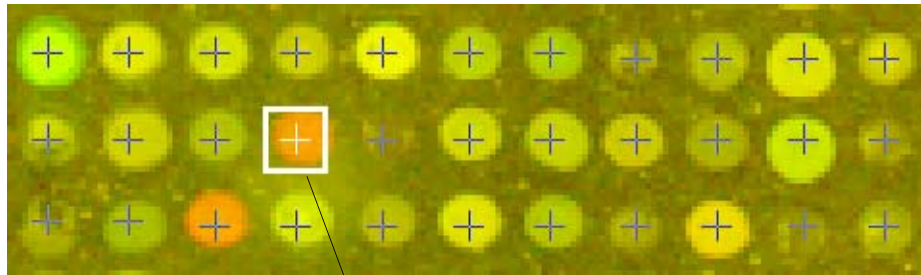**7** Select a feature from the list, and click **Go to**.

The software moves the Spot Navigator to that feature.

8 Click **Close**.

The software positions the Spot Navigator (square box) in the center of the Zoomed Spot View.
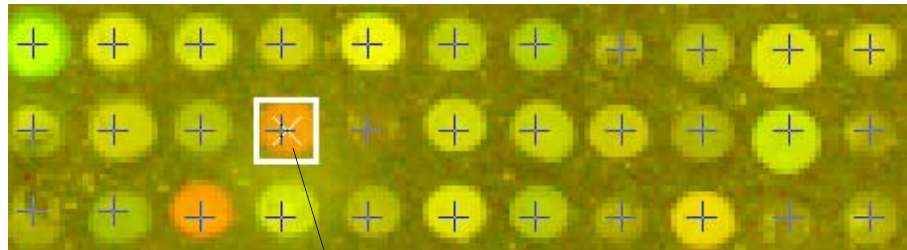
To position the spot centroid

Before you position the Spot Navigator to find the centroid, the position of the nominal spot centroid could look like this:

Nominal spot centroid (+) to be moved

• Move the Spot Navigator square containing the nominal spot centroid position to the assumed center of the spot.

The resulting position might look like this:

New estimated spot centroid (X) position;

These images represent non-Agilent microarrays. You will more likely need to position the spot centroid for non-Agilent microarrays.

When this altered grid file is used in Feature Extraction, the default spot finding algorithm will not attempt to move spots that have been manually located in the grid file.

To ignore spots
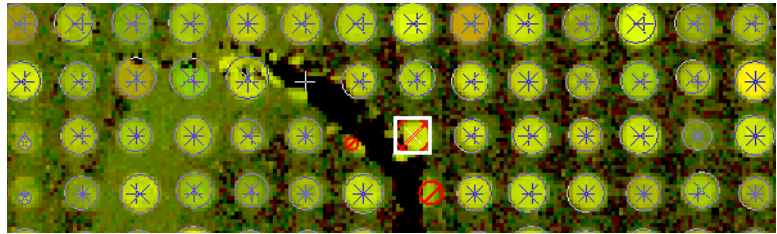
1 Position the **Spot Navigator** where you want to ignore a spot.
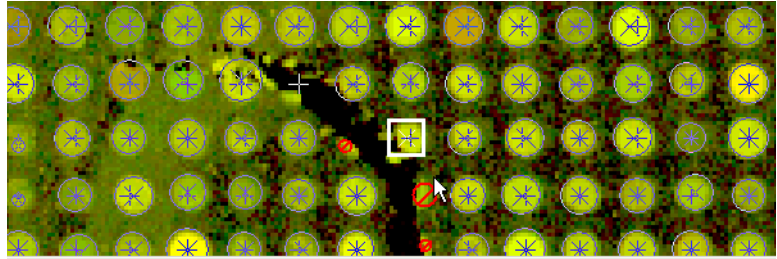
2 In the Grid Adjustment pane, click the field next to **Ignore** and then select **True**.

The software will not use this spot for any step in Feature Extraction.

To undo or redo spot position movements

- Click the **Undo** button, ⤺, to undo an action.
- Click the **Redo** button, ⤻, to redo an action.

To position spot centroids with the Grid Adjustment pane

When the Spot View appears, the Grid Adjustment tab is the default selection. You can position spot centroids by entering values for fields in the Grid Adjustment tab.

1 Click the **Grid Adjustment** tab, if necessary.

Figure 47   Spot Location Adjustment pane

To learn all the ways that you can move grids and spot centroids, see "Shortcuts to help you work with grids and find spots" on page 172.

2 Enter a value into any field that is available to you.

3 Press **Enter**.

Col and Row Numbers   Click the down arrows for Col and Row to select the column and row numbers for the spot centroid you want to position.

Center   Enter pixel numbers on X and Y axes for spot centroid location.

SpotDiam   Enter distance along X and Y axes in pixels for spot diameter.

ProbeName	Name of probe associated with selected spot; found in gene list
GeneName	Name of gene associated with selected spot; found in gene list
Ignore	Select true to ignore selected spot, or false to include it.

Refitting a grid

You may want to refit a grid under the circumstances mentioned in the table below. Alternate solutions to the problems are also described:

Table 5   Conditions for refitting grids

Grid Issue	Things to try before refitting the grid
Main grid is not properly placed.	Adjust the placement of the main grid. See "Step 3. Adjust the position of the main grid" on page 151.
Subgrids are not correctly spaced.	Adjust the spacing between subgrids. Assumption is that the subgrids are evenly spaced. See "Step 2. Get nominal subgrid spacing manually" on page 150 and "Step 4. Adjust the subgrid" on page 152.
Spot spacing appears off.	Adjust the spacing between the spots. "Step 1. Get nominal spot spacing manually" on page 148.
Average spot size is not correct.	Adjust the spot diameter. This diameter is used by the Calculate Spot Size and Centroids algorithm and the WholeSpot algorithm. See "Calculating spot size and spot centroids" on page 161 and "Locating spot centroids manually" on page 162.

CAUTION   When you refit a grid, any changes you made during manual gridding, if not saved as a grid file, are lost.
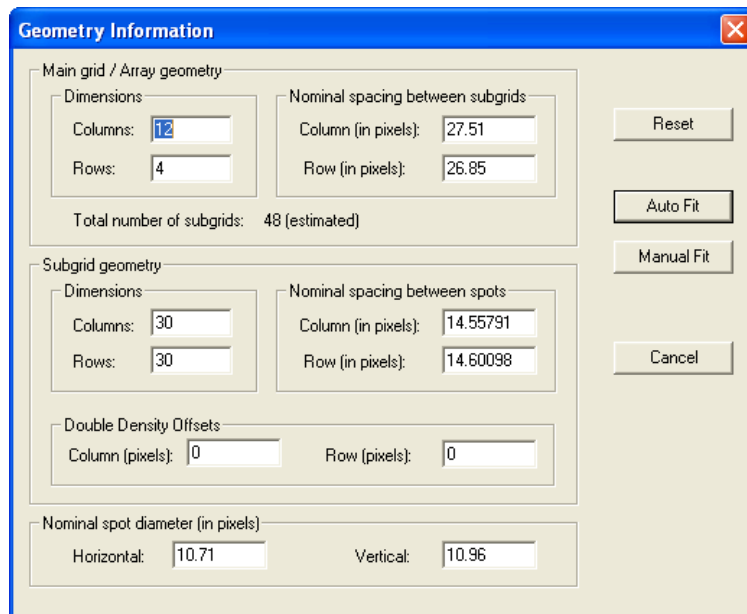
To learn about Geometry settings, see "Geometry information" on page 143.

To refit a grid, follow these steps.

1 Select **Edit > Refit Grid**.

The Geometry Information dialog box appears.

Click Reset to re-enter the original number before you changed it.

Figure 48 Geometry Information dialog box to refit Agilent grid

2 Change entries, and click **Auto Fit** or **Manual Fit**. A message will appear reminding you that refitting the grid will cause you to lose any changes you made that have not been saved in the grid file.

3 Click **Yes** to continue, or **No** to return to the Geometry Information dialog.

4 Once the grid has been refit, save the grid file to keep the changes made by the refitting procedure.

See "Saving a grid file" on page 160.

Changing default settings for Grid Editing

Before you begin to set up a grid, you can change the default settings that appear in the Grid Definition and Grid Adjustment panels on the main window when the grid mode is turned on.

1 Select **Tools > Preferences**.

2 Click the **Grid Editing** folder.
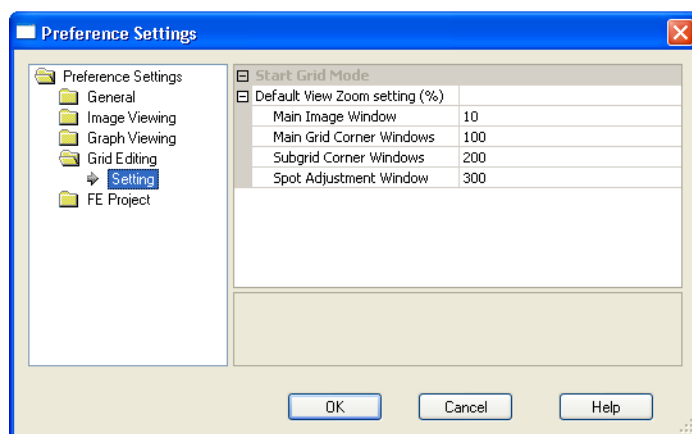
Figure 49   Preference Settings—Grid Editing selected

Default View Zoom setting   Click the cell next to an image window, then click the down arrow to select the zoom setting for each of the image windows listed.

Shortcuts to help you work with grids and find spots

There are several ways that you can select and move grids and spots. These are presented in Table 6. In addition, there are a number of **Ctrl** and **Alt** key operations that help you work with grids. These are presented in Table 7.

Table 6   Grid Actions

Location of actions	If you want to do this:	Do this on the Grid Adjustment dialog box:	Or, do this with the keyboard:	Or, do this with the mouse:
Main Grid	Set array label	Edit value, then press Enter.	NA	NA
	Move grid	Edit value, then press Enter.	Hold down the **Shift** key and select an arrow key.	Click the left mouse button and drag the cursor across the full window. Or, click the left mouse button in the corner window.
	Rotate grid	Edit value, then press Enter.	NA	Drag on corner in corner window.
Subgrids	Select subgrid[*]	Select column and row number from a choice list.	Use arrow keys or Page Up/Page Down (requires two rows or more) or Home/End	Left-click mouse in full view or corner view.
	Move subgrid	Edit location, then press Enter.	Hold down the **Shift** key and select an arrow key.	Drag the cursor across the full window. Or, click the left mouse button on the top left spot of the subgrid in the corner window.
	Rotate subgrid	Edit value, then press Enter.	NA	Drag on corner in corner window. Or, turn on automatic skew and rotate and then click the left mouse button on the upper left, upper right, and lower left spot of the subgrid in the corner window.

Table 6   Grid Actions (continued)

Location of actions	If you want to do this:	Do this on the Grid Adjustment dialog box:	Or, do this with the keyboard:	Or, do this with the mouse:
	Skew subgrid	Edit value, then press Enter.	NA	Click Skew Subgrid button, then drag on corner in corner window. Or, turn on automatic skew and rotate and then click the left mouse button on the upper left, upper right, and lower left spot of the subgrid in the corner window.
Spots	Select spot*	Select column and row number from a choice list.	Use arrow keys or Page Up/Page Down or Home/End	Left-click mouse in full view or corner view.
	Move spot centroid	Edit location, then press Enter.	Hold down the **Shift** key and select an arrow key.	Drag Spot Locator to position spot centroid.
	Flag spot	Mark the **Ignore this spot** check box, then press Enter.	NA	Double-click the right mouse button to toggle this flag.

*   These are the only grid actions that you cannot undo, redo or reset.

Table 7   Hot Key Actions for Grid Mode

If you want to do this:	Press this "hot key":
Turn Grid Mode On/Off	Alt-G
Select for Main Grid adjustment	Alt-M
Select for Subgrid adjustment	Alt-S
Select for Spot adjustment	Alt-P
Select to ignore a spot	Ctrl-G
Undo	Ctrl-Z
Undo All	Ctrl-A
Redo	Ctrl-Y

Table 7 Hot Key Actions for Grid Mode (continued)

If you want to do this:	Press this "hot key":
Redo All	Ctrl-D
Refit grid	Ctrl-T

4

Changing Protocol Settings

This chapter gives you background information on changing settings for the protocols used to perform Feature Extraction. The Feature Extraction protocols contain the parameter values for the algorithms that you specify for each step of the Feature Extraction process.

See Chapter 1 in the *Feature Extraction 10.10 Reference Guide* for a listing of the default settings and parameter values for the protocols shipped with the software.

Agilent Technologies

Changing protocol settings

Protocol templates

Agilent ships protocol templates for running Feature Extraction with various types of scan data. The name of each protocol template follows the format:

Agilent microarray type_software version_date

The table below lists the names of the templates that Agilent ships with Feature Extraction version 10.10, along with a description of changes from previous version. For a complete list of the default protocol settings, see the *Feature Extraction 10.10 Reference Guide*.

Table 8   Unremovable Protocol Templates

Protocol Template Name	Description
CGH_1010_Sep10	Recommended for all Agilent CGH microarrays.
	Updated from v. 10.9 to include:
	• 30 micron array format has been replaced by 30 micron feature size single pack and 30 micron multi pack array formats.
	• New parameter in Place Grid step: "Use central part of pack for slope and skew calculation?". Default is False, except for 30 micron single- and multi-pack array formats.
	• New parameter in Place Grid step: "Use the correlation method to obtain origin x of subgrids". Available only if Use central part of pack for slope and skew is set to True.
	• New parameter in the Flag Outliers step: "Report Population Outliers as Failed in MAGEML file". Default is False.
	• New parameter in the Correct Dye Biases step: "Use Dye Norm List". Default is Automatically Determine.
	• In the Correct Dye Biases step: Dye Normalization Probe Selection Method now has only two options: Use all Probes, and Use Rank Consistent Probes.
	• In the Correct Dye Biases step: Parameter "Max Number Ranked Probes" is now under the Normalization Correction Method.

Table 8   Unremovable Protocol Templates (continued)

Protocol Template Name	Description
ChIP_1010_Sep10	Recommended for all Agilent ChIP microarrays.
	Updated from v. 10.9 to include:
	• 30 micron array format has been replaced by 30 micron feature size single pack and 30 micron multi pack array formats.
	• New parameter in Place Grid step: "Use central part of pack for slope and skew calculation?". Default is False, except for 30 micron single- and multi-pack array formats.
	• New parameter in Place Grid step: "Use the correlation method to obtain origin x of subgrids". Available only if Use central part of pack for slope and skew is set to True.
	• New parameter in the Flag Outliers step: "Report Population Outliers as Failed in MAGEML file". Default is False.
	• New parameter in the Correct Dye Biases step: "Use Dye Norm List". Default is Automatically Determine.
	• In the Correct Dye Biases step: Dye Normalization Probe Selection Method now has only two options: Use all Probes, and Use Rank Consistent Probes.
	• In the Correct Dye Biases step: Parameter "Max Number Ranked Probes" is now under the Normalization Correction Method.
	• In the Generate Results step: Default for Type of QC Report is now CGH_ChIP.

Table 8   Unremovable Protocol Templates (continued)

Protocol Template Name	Description
GE1_1010_Sep10	Recommended for all Agilent 1-color gene expression microarrays using the latest wet lab protocols.
	Updated from v. 10.9 to include:
	• 30 micron array format has been replaced by 30 micron feature size single pack and 30 micron multi pack array formats.
	• New parameter in Place Grid step: "Use central part of pack for slope and skew calculation?". Default is False, except for 30 micron single- and multi-pack array formats.
	• New parameter in Place Grid step: "Use the correlation method to obtain origin x of subgrids". Available only if Use central part of pack for slope and skew is set to True.
	• New parameter in the Flag Outliers step: "Report Population Outliers as Failed in MAGEML file". Default is False.

Table 8   Unremovable Protocol Templates (continued)

Protocol Template Name	Description
GE2_1010_Sep10	Recommended for all Agilent 2-color gene expression microarrays using the latest wet lab protocols.
	• Updated from v. 10.9 to include:30 micron array format has been replaced by 30 micron feature size single pack and 30 micron multi pack array formats.
	• New parameter in Place Grid step: "Use central part of pack for slope and skew calculation?". Default is False, except for 30 micron single- and multi-pack array formats.
	• New parameter in Place Grid step: "Use the correlation method to obtain origin x of subgrids". Available only if Use central part of pack for slope and skew is set to True.
	• New parameter in the Flag Outliers step: "Report Population Outliers as Failed in MAGEML file". Default is False.
	• New parameter in the Correct Dye Biases step: "Use Dye Norm List". Default is Automatically Determine.
	• In the Correct Dye Biases step: Dye Normalization Probe Selection Method now has only two options: Use all Probes, and Use Rank Consistent Probes.
	• In the Correct Dye Biases step: Parameter "Max Number Ranked Probes" is now under the Normalization Correction Method.

Table 8   Unremovable Protocol Templates (continued)

Protocol Template Name	Description
GE2-nonAT_1010_Sep10	This is the recommended protocol for non-Agilent microarrays.
	Updated from v. 10.9 to include:
	• 30 micron array format has been replaced by 30 micron feature size single pack and 30 micron multi pack array formats. • New parameter in Place Grid step: "Use central part of pack for slope and skew calculation?". Default is False, except for 30 micron single- and multi-pack array formats. • New parameter in Place Grid step: "Use the correlation method to obtain origin x of subgrids". Available only if Use central part of pack for slope and skew is set to True. • New parameter in the Flag Outliers step: "Report Population Outliers as Failed in MAGEML file". Default is False. • New parameter in the Correct Dye Biases step: "Use Dye Norm List". Default is Automatically Determine. • In the Correct Dye Biases step: Dye Normalization Probe Selection Method now has only two options: Use all Probes, and Use Rank Consistent Probes. • In the Correct Dye Biases step: Parameter "Max Number Ranked Probes" is now under the Normalization Correction Method.

Table 8   Unremovable Protocol Templates (continued)

Protocol Template Name	Description
miRNA_1010_Sep10	Recommended for all Agilent miRNA microarrays using the latest wet lab protocols.
	Updated from v. 10.9 to include:
	• 30 micron array format has been replaced by 30 micron feature size single pack and 30 micron multi pack array formats.
	• New parameter in Place Grid step: "Use central part of pack for slope and skew calculation?". Default is False, except for 30 micron single- and multi-pack array formats.
	• New parameter in Place Grid step: "Use the correlation method to obtain origin x of subgrids". Available only if Use central part of pack for slope and skew is set to True.
	• New parameter in the Flag Outliers step: "Report Population Outliers as Failed in MAGEML file". Default is False.

Both the protocol parameters and default values may change over time. To ensure that you have the latest protocols, you must set up eArray login and let Feature Extraction automatically check the eArray server for the protocol updates every time the software starts. See "Setting up eArray login for automatic updates" on page 36.

To create a new protocol from a protocol template

See Chapter 5, "How Algorithms Calculate Results" in the Feature Extraction 10.10 Reference Guide for detailed information on how the protocol steps and algorithms work.

Each protocol contains protocol steps that represent Feature Extraction algorithms. In the FE Protocol Editor, you make changes to the parameter values for each of the steps.

After you open the FE Protocol Editor for a specific protocol, you can change settings, but you must then save the protocol to a new name. The process is as follows:

1 To open a protocol.

2 To view or change the protocol properties.

3 To change the protocol steps to run.

4 To change the settings for each protocol step.

5 To save a protocol with a new name.

The following sections present instructions for creating protocols from templates and tasks for changing settings.

Creating a new protocol from an existing protocol

To create a protocol for a specific type of microarray, you *must* use an existing protocol for the same type of microarray as a template.

For example, if you want to create a new protocol to run a gene expression 2-color microarray, start by editing an Agilent-created protocol for gene expression 2-color microarrays.

You can track what the original Agilent-created protocol is by looking at the **Derived From** field of the general section of the new protocol. See "To change the protocol steps to run" on page 187.

CAUTION	Existing protocols can be used as templates for new protocols to provide both visible and **hidden** settings whose values are specific to the type of microarray. Although you can change the visible settings of any two protocols of different types to *appear* identical, you **cannot change the hidden settings** that distinguish these protocols from one another.

To open a protocol

You can open the FE Protocol Editor in one of three ways:

- In the **Protocol Browser** double-click the protocol that you intend to edit, or right-click the protocol and select **Properties**.

- In Project Explorer, either double-click the protocol assigned to an extraction set, or right-click the protocol and select **Properties**.

- Click the **Extraction Configuration** tab, and in the **Protocol** column double-click the protocol that you intend to edit.

Regardless of the protocol, the first screen that appears is by default the screen that was last displayed before a protocol was closed or saved.
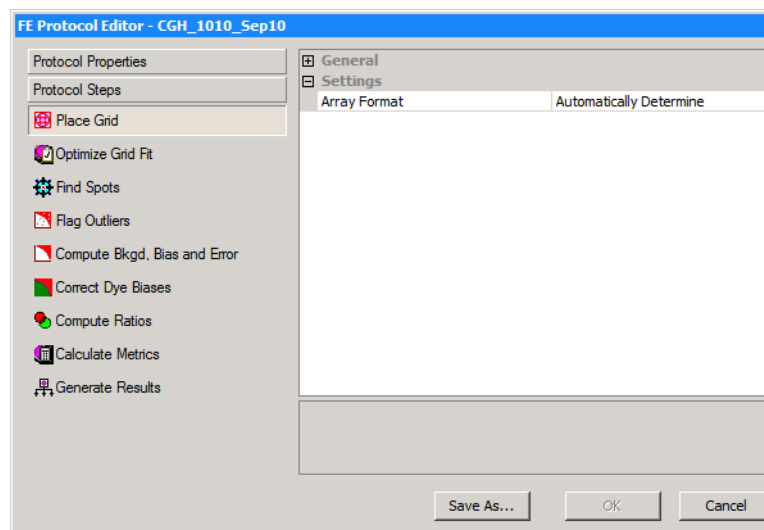

Figure 50   FE Protocol Editor – Protocol Steps

To view or change the protocol properties

The General section of the Protocol Properties page lists fields to identify the protocol and fields to let you remove the protocol or make the protocol read-only.

• If necessary, in the FE Protocol Editor click the **Protocol Properties** button to see the **General** section.
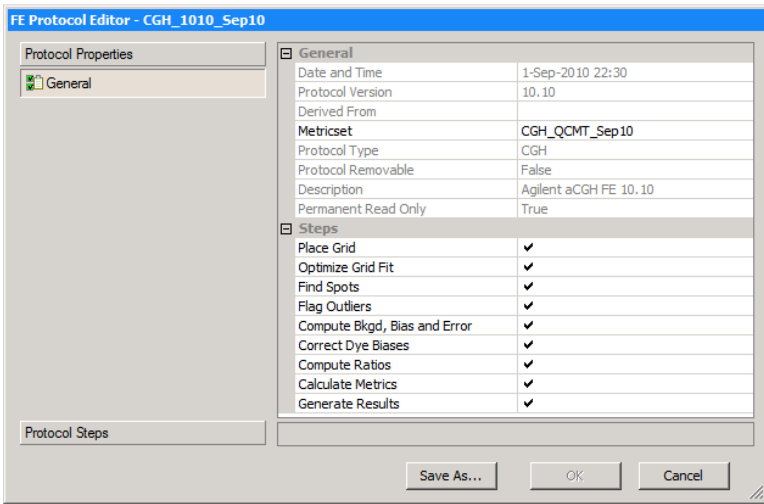

Figure 51   FE Protocol Editor – Protocol Properties tab

Date and Time   Timestamp for the creation of the protocol.

Protocol Version   Version of the original Agilent-created protocol, corresponding to software version with which it was shipped.

Derived From   Name of original Agilent-created protocol from which the current protocol is derived; blank until you exit and reopen modified protocol.

Metricset   Name of the QC metric set that is to be used with this protocol to generate metrics (statistics) shown in the QC Report and Stats table.

Protocol Type	Corresponds to type of microarray for which the protocol is to be used for Feature Extraction: 2-color gene expression, 1-color gene expression, CGH, ChIP, or miRNA data.
Protocol Removable	Describes if the protocol is removable from the database. User-created protocols are removable. Agilent-created protocols are not removable.
Description	Description of the type of microarray and format for which the protocol was designed
Permanent Read Only	True indicates that the protocol cannot be edited. Any changes made to the protocol must be saved with a different name.
	False indicates that the protocol can be edited. Any changes made to the protocol can be saved with the existing name.

To change the protocol steps to run

If there is no protocol on your system with the steps you want Feature Extraction to use, you can open a protocol of the appropriate type and change the protocol steps.

1 If necessary, in the FE Protocol Editor click the **Protocol Properties** button to see a list of the protocol steps on the right.

2 Mark each check box to enable a step that you want to run. Clear each check box to disable a step that you do not want to run.

Refer to Figure 51 to see the FE Protocol Editor.

Protocol steps for GE 2-color, CGH, and ChIP images

Below is a description of each of the protocol steps for GE 2-color, CGH, and ChIP images, as seen in Figure 51, in the order that the software runs them.

Place Grid
- For Agilent microarrays using a grid template, automatically places a grid on the microarray.

- For any microarray loaded with a grid file that you saved during Grid Mode (i.e. interactive gridding), automatically calculates centroid positions. Any spots that were manually adjusted are left unchanged.

- For Agilent microarrays, the software performs a background peak shift that helps find peaks of features that are close to the background. See the *Feature Extraction 10.10 Reference Guide* for details.

- For any microarray, user can select to use regions near to the center of packs for slope and skew calculations. This option is of use in cases where pack edges have dim spots and are failing to grid. When this is selected, the user can select to have the software to perform one extra step of correlation following the projection data analysis to get the origin.

Optimize Grid Fit
- For microarrays with 30- or 65-micron feature size, iteratively adjusts the grid by looking at spots in the corners of the microarray to improve overall image registration.

Find Spots
- The spot centers are located and/or optimized starting with the spot nominal positions on the grid.

- From the located spots, the features are defined with either a CookieCutter method or a WholeSpot method.

- The local background is also defined with a radius method.

- Outlier pixels (too low or high intensity) are then removed from the pixel populations that defined the features and backgrounds.

- Statistics, such as the mean, median and standard deviation (SD), are calculated on the remaining inlier pixels within the feature and background.

- Features with a large number of saturated pixels are flagged.

- The protocol determines whether the mean or median signals are used for further analysis.

Flag Outliers
- Features are flagged as non-uniformity outliers if their pixel distributions are outside the threshold limits.

- Features that have replicates of the same probe sequence and background regions are flagged as population outliers if their signal distributions are outside the set range.

Compute Bkgd, Bias, and Error
- If background subtraction is selected, background signal is subtracted from raw signal for each feature using a local or global background method.

- A statistical test is performed to assess if the feature signal is significantly different from the background signal. This test uses either the error model or pixel statistics, as indicated in the protocol choice "Significance".

- Raw signals or background-subtracted signals can be corrected for systematic gradients, that affect low signals especially, by using additive detrending (i.e. spatial detrending).

- The error is calculated at this point, using the choice between the Universal error model or the larger of the error estimates (i.e. most conservative) from the Universal error model and the propagated error.

- A multiplicative detrending algorithm is also used to correct for gradients proportional to the signal.

- Surrogates, based upon background noise, can be used as processed signals for features that have low-intensities, near the background noise level.

Correct Dye Biases
- The method for how a dye normalization gene list is selected is set.

- Differences in the red and green channel signals caused by different efficiencies in labeling, emission and detection are estimated and corrected.

- Features to be used for measuring dye bias are selected based on a probe selection method chosen.

- A dye normalization curve fit method is selected and used to fit the data for measuring dye bias. (SNP probes are not included in dye normalization calculations.)

Compute Ratios
- Log ratios of the red and green processed signals (i.e. background-corrected and dye-normalized) are calculated, as well as a log ratio error and a p-value for each feature. For gene expression 2-color arrays, these calculations assess the confidence you can have that the gene is or is not differentially expressed.

Calculate Metrics
- In this step, the Stats table is generated and QC metrics are calculated for the QC Report.

  You must leave this option turned on to generate a complete Stats table and QC report. Some values will be missing if this option is turned off.

- The type of protocol you select determines the type of QC report that is generated.

  For example, a CGH protocol generates a CGH QC Report, and a 1-color protocol with spike-ins selected generates a 1-color QC Report with spike-ins.

Generate Results
- Results selected for the project are generated.

  In the Protocol Editor you can select to generate one file for the three tables (FEPARAMS, STATS, FEATURES) of text results or three separate files. You can set the compression for the jpeg file.

Protocol steps for GE 1-color and miRNA images

GE 1-color   Feature Extraction analyzes GE 1-color images using the same steps listed for GE 2-color images except it does not use the Correct Dye Biases and Compute Ratios steps.

miRNA   The miRNA analysis also does not correct for dye biases nor compute ratios. Instead, the analysis estimates the expression level by sampling multiple probes with multiple features per probe and reporting a total gene signal (counts/pixel), independent of microarray design, rather than a processed signal.

The protocol, then, does not contain the Correct Dye Biases step nor the Compute Ratios step, but it does contain a **MicroRNA Analysis** step before the Calculate Metrics step.

To change the settings for each protocol step

After you change the protocol steps that you want to run, you may want to change the default settings and parameter values for a specific protocol step.

1 Click the **Protocol Steps** tab to see a list of the protocol steps, or Feature Extraction algorithms, on the left.

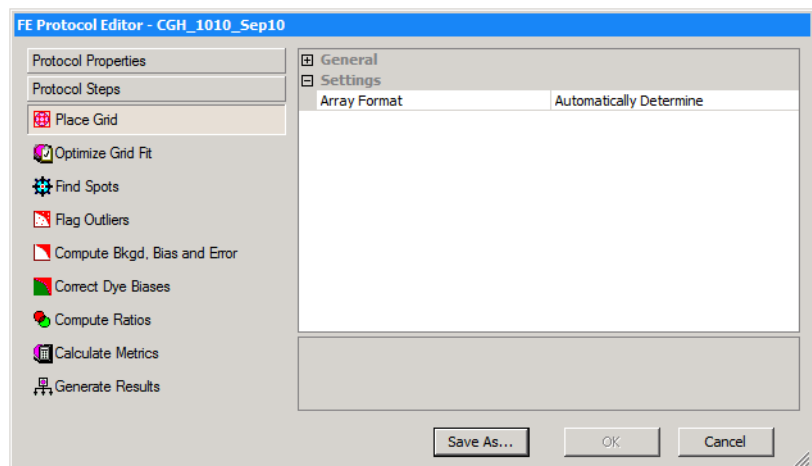2 Click the protocol step button whose settings you intend to change.

Figure 52   FE Protocol Editor with Protocol Steps on left

See the remaining sections in this chapter to learn how and why you change parameters for each protocol step.

To save a protocol with a new name

After you make any change to a protocol, you must save the protocol to a new name.

1 Make the changes you intend.
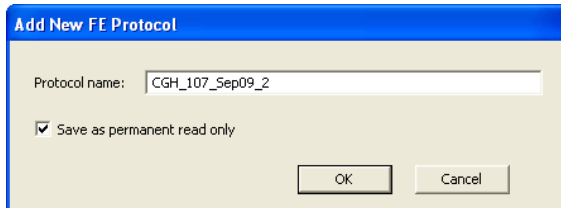
2 Click **Save As**.

Figure 53   Add new protocol dialog box

3 Enter a **Protocol name**.

4 If you want to make the protocol editable, clear the check box **Save as permanent read only**.

For standard protocols that you use frequently, you may want to save these protocols as permanent, read-only protocols so that others cannot alter them.

5 Click **OK**.

Changing Protocol Step 1: Place Grid

The Place Grid algorithm initiates Feature Extraction by placing a grid on the microarray image. The grid comes from either the grid template or the grid file associated with the image file in the project.

To access the settings to place a grid

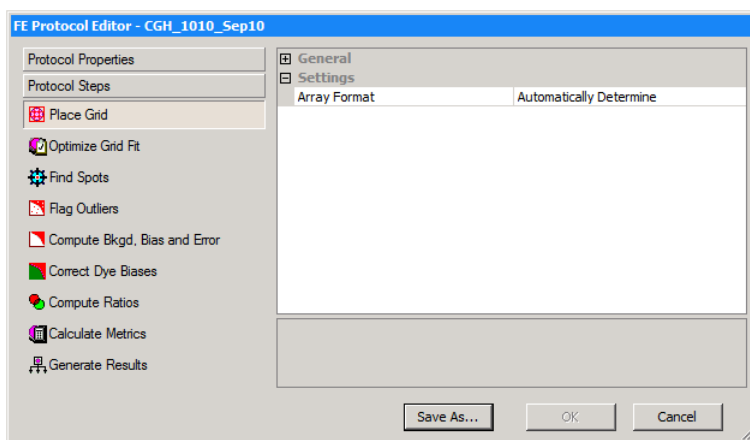• Click the **Place Grid** tab in the FE Protocol Editor, if not already there.

Figure 54   FE Protocol Editor — Place Grid selected

To select the microarray format

This topic describes how to select an array format if needed. You typically do not need to do this step, as the software automatically determines which array format and scan type is used.

Once you change to a specific array format, the protocol loses its ability to be general and will only work for that specific format.

1 Click the cell to the right of Array Format.

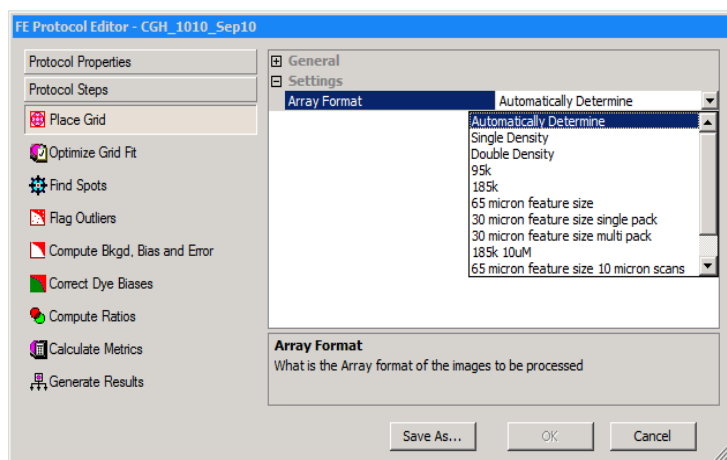**2** Click the down arrow, and select a microarray format from the list.

Figure 55   Place Grid — Array Format list

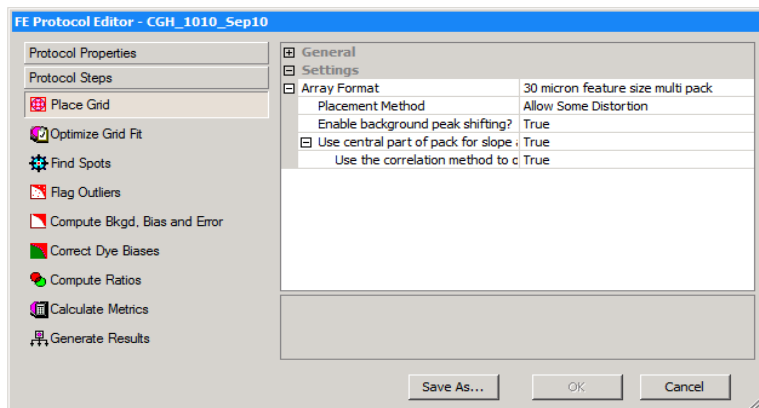The Placement Method option now appears. See Figure 56 on page 195.

Figure 56   Place Grid — 30-micron multi pack Array Format selected,
Placement Method

After you select the format, you can select a method for
placing the grid.

Protocols can automatically detect the array format (65-
micron feature size or 30-micron feature size). The protocol
automatically makes different parameter choices depending
on the format and type. These choices are transparent to
you and are primarily in the image processing (Place Grid
and Find Spots).

With this feature, you do not need different protocols for
different array formats. These protocols contain the correct
parameter choices for each and use the set that matches the
format.

Information about which parameters are affected and which parameters are chosen for each format type can be found beneath any option with a choice "automatically determine". If you believe an incorrect automatic choice was made, you can select a specific format. However, this is not a normal or necessary step.

To select a method for placing the grid

1 Click the cell next to **Placement Method**, and click the down arrow.

2 Select one of the two options listed below.

Allow Some Distortion   Allows some variance in the feature spacing, subgrid spacing and the skew. All of the protocol templates have this default setting.

Place and Rotate Only   Only allows rotation of the grid and no changes to feature spacing, subgrid spacing or the skew.

To select to enable background peak shifting

1 Click the cell next to **Enable background peak shifting?** and click the down arrow.

2 Select **True** or **False**.

If you select True, the background pixel value is obtained using the histogram of the image. All the pixels in the image that belong to background are reassigned a value of zero. Note that this assignment is temporary and does not change the actual image data for subsequent steps.

To select to use central part of pack for slope and skew calculations

This option lets you choose the region to use for slope and skew calculation during subgrid placement.

1 Click the cell next to **Use central part of pack for slope and skew calculations?** and then click the down arrow.

2 Select **True** or **False**.

When set to True, regions near to the center of packs are used. This option is of use particularly in cases where pack edges have dim spots and are failing to grid. (This is the default setting for 30 micron single- and multi-pack array formats.)

When set to False, the regions near to the edges of packs are used. Selecting this option uses the same parameters as Feature Extraction version 10.7/10.9 or earlier.

To select to use the correlation method to obtain origin x of subgrids

This option lets you choose the technique to use to determine X location of origin of subgrids.

1 Click the cell next to **Use central part of pack for slope and skew calculations?** and click the down arrow.

2 Select **True**.

3 Click the cell next to **Use the correlation method to obtain origin x of subgrids**? and then click the down arrow.

4 Select **True** or **False**.

When set to True, the software performs one extra step of correlation following the projection data analysis to get the origin. This option is of use particularly in cases where pack edges have dim spots and are failing to grid.

When set to False, the results obtained from the projection data analysis are used to estimate the origin. This is how the origin is estimated using Feature Extraction version 10.7/10.9 or earlier.

Changing Protocol Step 2: Optimize Grid Fit

The Optimize Grid Fit step is a post grid placement process to improve the grid registration. Leveraging from the spot finder algorithm, this step examines the spots in the four corners of the microarray to iteratively adjusts the grid for best fit.

To access the settings to optimize the grid fit

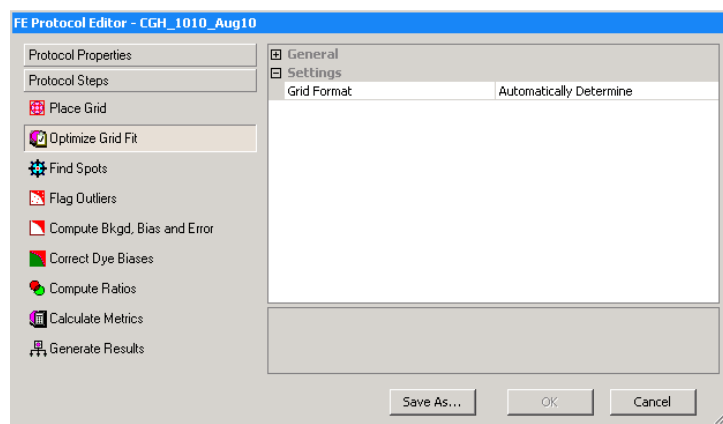- Click the **Optimize Grid Fit** tab in the FE Protocol Editor.

Figure 57   FE Protocol Editor — Optimize the Grid Fit selected

To select the grid format

1 Click on the cell next to **Grid Format**.

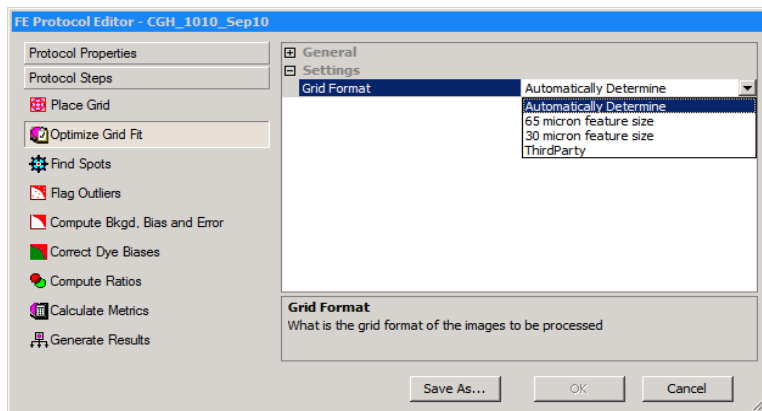**2** Click the down arrow, and select from the list of grid formats.

Figure 58   Optimize Grid Fit — Grid Format list

If you select either 65-micron or 30-micron feature size, the **Iteratively Adjust Corners?** option appears. See Figure 59 on page 201.
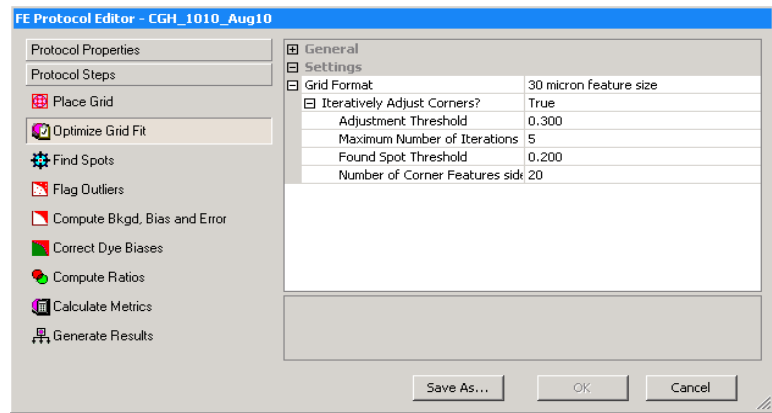
Figure 59   Optimize Grid Fit — 30-micron Feature Size selected,
Iteratively Adjust Corners

To change the settings to iteratively adjust corners when grid format is selected

1 Click the cell next to **Iteratively Adjust Corners?**.

2 Either select **True** or **False**.

3 If you select True, you can change any of the default parameters that you need to.

When set to True, the Interactively Adjust Corners method runs the spot finder algorithm in the corners to see how well the grid is placed on the microarray and iteratively adjusts the grid based upon the results.

Adjustment Threshold	The grid is adjusted based upon the difference between the grid position and the spot position if the absolute average difference is greater than this value.
Maximum Number of Iterations	This is the maximum number of times the spot finder algorithm runs to adjust the grid for better grid fitting in the corner spots.
Found Spot Threshold	For each corner, the grid is adjusted only if this fraction or more of the features in that corner are considered found by the spot finder algorithm.
Number of Corner Features Side Dimension?	This specifies the number of features (i.e. spots) on each side of the square in each corner of the microarray to be used to calculate the average difference.
	The default number is 20 for the 30- and 65-micron feature size microarrays. This means each corner of the microarray has a square area of 400 (i.e. 20x20) features that are calculated.

Changing Protocol Step 3: Find Spots

See Chapter 1, "Default Protocol Settings", of the Feature Extraction 10.10 Reference Guide for a setting comparison between the various microarray formats.

After Feature Extraction places the grid, it is ready to locate the spot centers and define the features and local background regions for each spot. The options available and default values for each setting depend on the microarray format selected in Step 1: Place Grid.

To access the settings to find spots when spot format is automatically determined

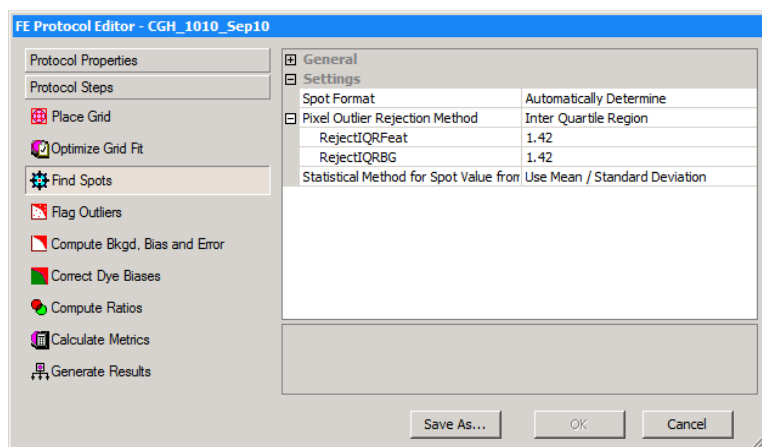- Click the **Find Spots** tab in the FE Protocol Editor.

Figure 60   FE Protocol Editor — Find Spots selected

A *spot* is a circular or amorphous area on the microarray containing a hybridized sample.

A *feature* is defined as the pixels in either the center portion of a spot or in the whole spot, depending on the method you choose to define the feature.

When the spot format is automatically determined (default setting), these are the only settings available for change:

• Select the statistical method to reject outlier pixels from features and local background before Feature Extraction calculates feature and background statistics.

• Select whether to use mean or median for data analysis

To select the statistical method to reject pixel outliers

1 Click the cell next to **Pixel Outlier Rejection Method**, and click the down arrow.

2 Select one of these three choices:

Standard Deviation   This calculation rejects outliers outside of a specified number of standard deviations from the mean of the intensity for all pixels. If selected, you can enter the number of standard deviations (**Feature SD** and **Background SD**).

Inter Quartile Region   This calculation rejects outliers based on the Interquartile Range (IQR). See the *Feature Extraction 10.10 Reference Guide* for how this calculation works. If selected, you can enter the IQR factor for both the feature and background (**RejectIQRFeat** and **RejectIQRBG**).

None   If selected, no pixels are rejected.

Feature Extraction rejects outlier pixels after calculating statistics on the intensities of all the pixels of a feature or local background region for each color. If a pixel is rejected in one color, it is rejected in both colors.

To select whether to use mean or median for data analysis

1 Click the cell next to **Statistical Method for Spot Value from Pixels**, and click the down arrow.

2 Select one of these choices to be used in the next step of data analysis:

Mean   This selection uses the mean and standard deviation.

Median   This selection uses the median and normalized IQR for the next steps of data analysis.

During the background correction stage the background can be subtracted from either the mean signal or the median signal. This option lets you decide which signal type to use for downstream analysis.

To access the settings to find spots when spot format is selected

1 Click the cell to the right of the **Spot Format**.

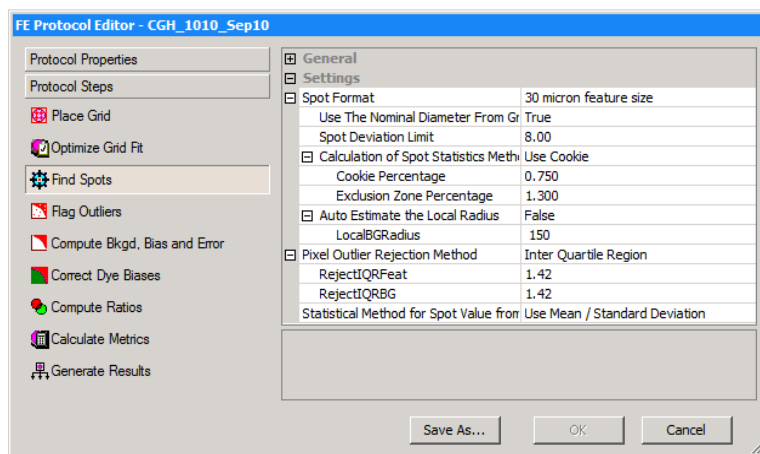**2** Click the down arrow, and select from the list of spot formats.

Figure 61   Find Spots — 30-micron Spot Format selected

In this protocol step you can also change the values for 3 additional sets of parameters associated with Feature Extraction processes to find spots once you have selected a spot format:

• Change values to find and size spots.

• Select a spot statistics method to define features.

• Select to autoestimate the local radius.

To change values to find and size the spots.

1 Click the cell next to the names of the following parameters.

2 Either select **True** or **False**, or enter a value, whichever is applicable.

Use the Nominal Diameter from the Grid Template If you select True, the nominal diameter of each spot is obtained from the grid template. If you select False, the nominal diameter that is the starting spot size assumption for the Spot Finding algorithm is obtained from the grid placement algorithm.

Spot Deviation Limit Enter a value that defines the distance the actual centroid may move relative to its nominal grid position (stated as a multiple of nominal radius). This parameter appears in the FEPARAMS table as SpotAnalysis_kmean_cen_reject.

To select a spot statistics method to define features

1 Select either **Use Cookie** or **Use Whole Spot**.

Cookie Method   The Cookie method locates an ellipse (usually a circle) over the centroid of each spot with a fixed radius entered in the protocol and defines the following regions represented in the figure below:

The Cookie method is usually used for Agilent microarray spots because of their uniformity.
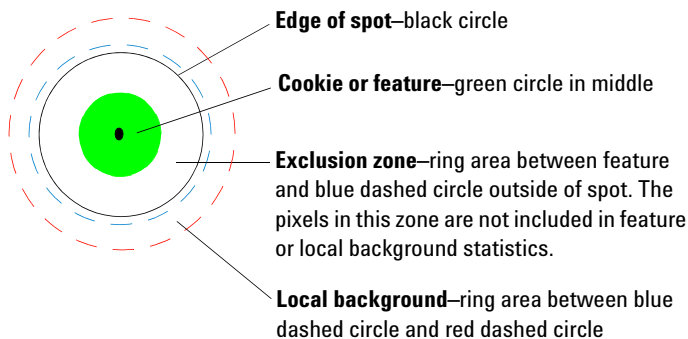

Edge of spot–black circle

Cookie or feature–green circle in middle

Exclusion zone–ring area between feature and blue dashed circle outside of spot. The pixels in this zone are not included in feature or local background statistics.

Local background–ring area between blue dashed circle and red dashed circle

Figure 62   Feature and background definitions – Cookie method

Whole Spot Method   This method uses a detection technique based on a noise model to define the feature and exclusion zone. This method performs the spot size calculation first and defines the feature as the whole spot from the x and y diameters.
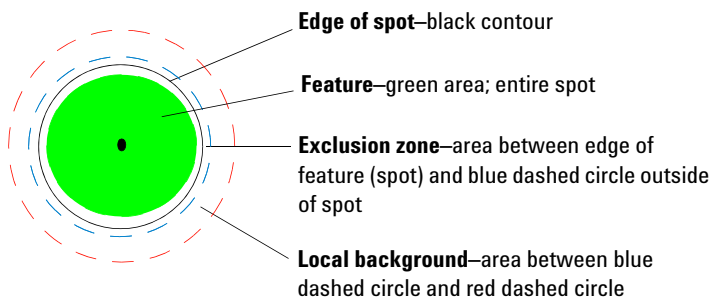

Edge of spot–black contour

Feature–green area; entire spot

Exclusion zone–area between edge of feature (spot) and blue dashed circle outside of spot

Local background–area between blue dashed circle and red dashed circle

Figure 63   Feature and background definition – Whole Spot method

2 If you selected **Use Cookie**, change the **Cookie Percentage** and/or the **Exclusion Zone Percentage**, if necessary.

If you selected **Use Whole Spot**, change the **Exclusion Zone Percentage**, if necessary.

Cookie Percentage   Percentage of the nominal radius that is the cookie radius.

Exclusion Zone Percentage   Percentage of the nominal radius that is the exclusion zone radius.
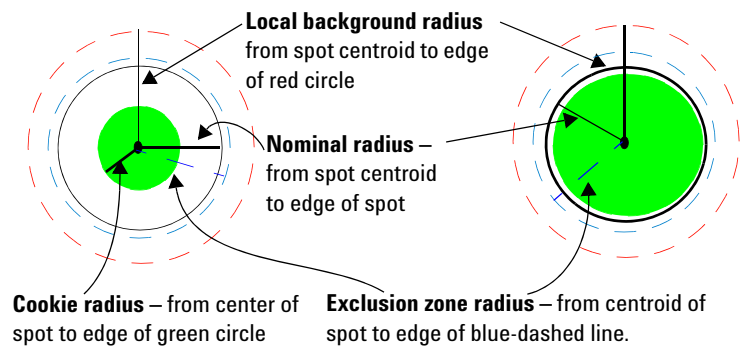

Local background radius
from spot centroid to edge of red circle

Nominal radius –
from spot centroid to edge of spot

Cookie radius – from center of spot to edge of green circle

Exclusion zone radius – from centroid of spot to edge of blue-dashed line.

Figure 64   Definitions of different radii

See the Feature Extraction 10.10 Reference Guide for more information.

The radii depicted in Figure 64, "Definitions of different radii," on page 209 are calculated differently for the two methods.

To select to autoestimate the local radius to define local background

This radius method estimates background that is local to a feature of interest.

1 Click the cell next to **Autoestimate the local radius**.

2 Select **True** or **False**.

For non-Agilent microarrays when there is a big difference in spot sizes, change this setting to False and make sure that the number you enter is big enough to accommodate the largest spot on the microarray.

3 If you select False, enter a larger radius if you want to include more background pixels or a smaller one if you want to include fewer.

If you select True, the software automatically estimates the radius to use to estimate the local background.

This approach eliminates any dependence of the local background computation on distance between spot-centroids along both axes. This ensures elimination of cross-contamination between feature and background signals, especially in closely packed microarrays.

See the Feature Extraction 10.10 Reference Guide for details on this calculation.

The default radius produces a local background calculation around only one spot (Self-radius). If you want to include more background, you can increase the radius to include the background around the spot's nearest neighbors (NN radius).

Final calculations for protocol step to find and measure spots

Final statistics

The final statistics calculated for all colors of each feature and local background, based on the remaining inlier pixels, are the average, standard deviation, median, IQR, and number of pixels. In addition, the pixel correlation between the red and green channels is calculated.

Pixel saturation

Saturation is defined as the maximum allowed intensity value for a pixel, Its value depends on the make and model of the scanner.

Pixel saturation is also tested during calculation of the feature statistics. For the Agilent Microarray Scanner system, saturation is defined as an intensity value of 65535 minus the dark offset from the scanner. If more than 50% of the pixels remaining after outlier rejection are saturated, Feature Extraction sets a saturation flag.

With XDR extractions, the saturation limit could be as much as 20x higher than 65535. The array will saturate before it gets to 20x of 65535. The calculated saturation value based upon the XDR fit could be as high as 1,310,000 counts, but no biological signals are that high.

20-bit scans also have a high saturation value, one close to 1,048,576 counts.

Propagation of Find Spot errors to other algorithms

The measurement errors from the calculation of these statistics are propagated to the next steps in Feature Extraction according to standard error propagation techniques. For example, the errors for the signal average for the feature and local background regions are propagated to the background subtraction algorithm.

Changing Protocol Step 4: Flag Outliers

Not every feature is created, hybridized and scanned perfectly. Some backgrounds contain artifacts. Therefore, you can flag those features or local backgrounds that may represent misleading or erroneous data.

Although the grid tool of the Feature Extraction software lets you manually examine scanned microarray images and flag anomalous features, this task becomes exceedingly tedious and subjective for thousands of features on a microarray. This algorithm automatically finds all the anomalous features on the microarray.

To access the settings to flag outliers

- Click the **Flag Outliers** tab to change settings for flagging abnormal features and backgrounds.
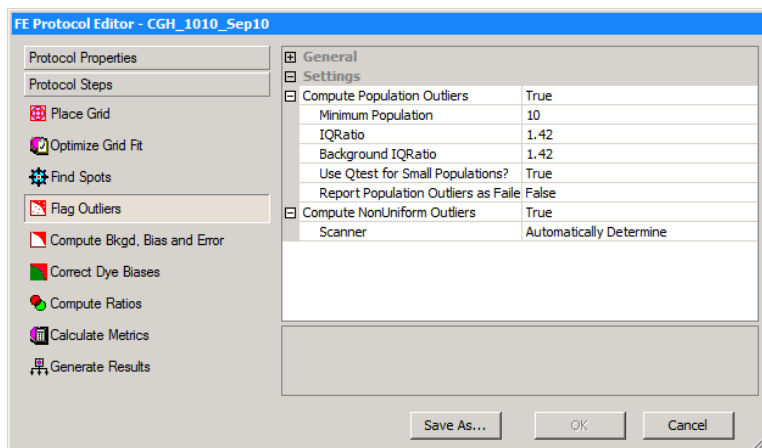
Figure 65   FE Protocol Editor — Flag Outliers selected

You can change two types of settings for this algorithm:

- Change settings to flag population outliers.

  With this option you can flag features and local background regions that have intensities beyond the confidence limit for the distribution of the intensities for the replicate features and local background regions on the microarray.

- Change settings to flag non-uniform features and background region (outliers).

To change settings to flag population outliers

A type of outlier, called a "population outlier," can be detected if there are enough replicates to perform population statistics on the signal intensities of the features or the signal intensities of the background regions.

For example, there are a number of control features on a typical Agilent microarray (typically, over 100 features) and thousands of local background regions. Out of this population, outliers can be rejected using box plot analysis.

1 Click the cell next to **Compute Population Outliers,** and click the down arrow.

2 Select **True** or **False**.

3 If you select **True**, change any of the default parameters that you need to.

The Flag Outliers tab contains values for the minimum population size to be considered for population outlier flagging, as well as a cutoff range. If a feature or local background region signal lies outside of this range, Feature Extraction flags that feature or local background region as a population outlier.

Minimum population   This is the minimum number of replicates needed to perform the population outlier analysis. Although 10 is the default number of replicates, you can enter as low as 5 replicates.

To take advantage of the replicated probes in statistical calculations, Agilent uses the default threshold of 10 because its catalog microarrays have 100 probes that are replicated 10 times on the microarray. The default value for non-Agilent microarrays is 15.

IQRatio   This formula determines the cutoff for box plot analysis. The 1.42 x IQ Range (Interquartile Range) encompasses 99% of normal distribution. The Interquartile Range is the 75th percentile - 25th percentile. This calculation is the same as that for rejecting pixels that are of too low or too high intensity. In this case, however, the outlier features are flagged, not removed.

Background IQRatio   This option uses the IQRatio formula for estimating the background region outliers.

Use Qtest for Small Populations?   When the number of replicates falls below the minimum number specified in the protocol, a Q-test can be used to estimate the number of population outliers instead of the IQRatio. See Chapter 5 of the *Feature Extraction 10.10 Reference Guide* to learn more about Q-test calculations.

Report Population Outliers as Failed in MAGEML file   When this option is set to *True*, any feature that is flagged as a population outlier is reported as *Failed* in the MAGE-ML output file.

To change settings to flag non-uniform outliers

The Agilent Microarray system includes Agilent microarrays, label/hyb/wash protocols and reagents, scanner and Feature Extraction algorithms.

The non-uniformity outlier algorithm flags anomalous features or local backgrounds based upon statistically significant deviations from a noise model that makes use of the known noise characteristics of the Agilent Microarray system.

You do not typically need to do this step, as the software automatically determines the scan type and sets the parameters appropriately. To view or change the parameters

that are automatically set, select a specific scan type. However, if you change the parameters, the protocol is no longer general and can only be used with that scan type.

1 Click the cell next to **Compute NonUniform Outliers,** and click the down arrow.

2 Select **True** or **False**.

"True" is the default setting for all Agilent-created protocols. If True is set, move on to the next step.

3 Click the cell next to **Scanner,** and click the down arrow.

4 Select either **Automatically Determine** or **Agilent Scanner**.

The settings change depending on your selection.

To learn more about how the algorithm calculates the terms in the polynomial equation below, see the Feature Extraction 10.10 Reference Guide.

5 Click the cell next to **Automatically Compute OL Polynomial Terms**.

6 Select **True** or **False**.

True is default setting. A different set of parameters appears for a True or a False setting.

7 Enter the values for the parameters.

Parameters for a "True" setting
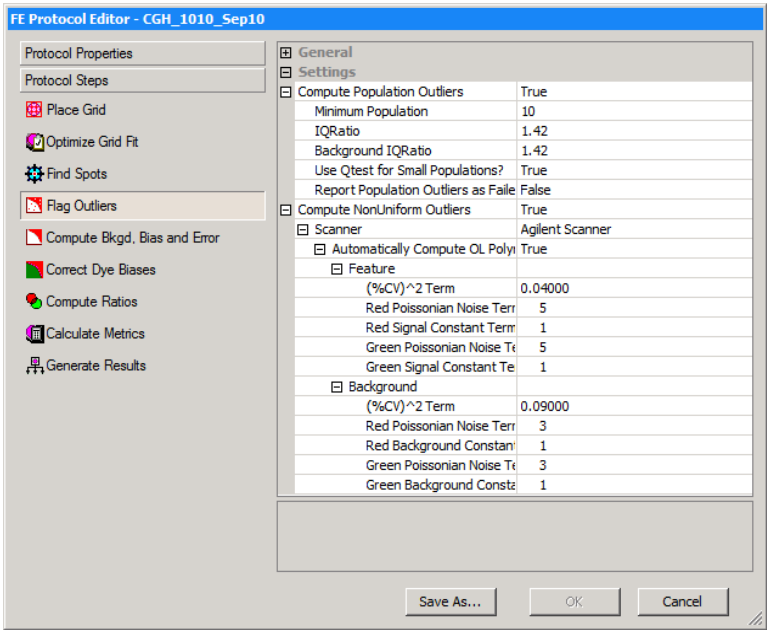
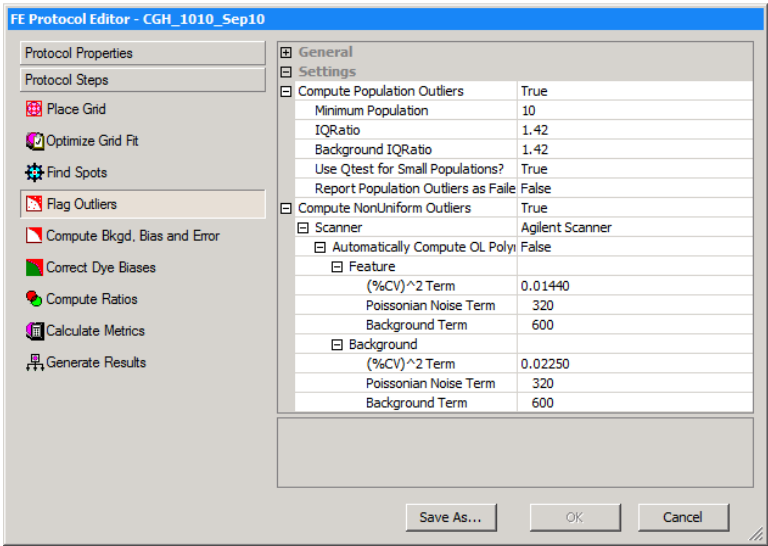**Figure 66** Flag Outliers — Agilent Scanner selected,
Automatically Compute OL Polynomial Terms: True

The polynomial equation for the noise model is still used, but rather than entering values for the A, B and C terms as in the "False" case, you enter the values for the A (%CV)^2 term and multipliers for the B and C terms in both color channels (2-color) or one channel (1-color).

(%CV)^2 Term (A) This term is the square of the CV (the constant coefficient of variation), or the intensity-dependent variance.

Red Poissonian Noise Term (B) Multiplier The B term for the red channel is equal to this multiplier times the net signal at the 25% percentile of a histogram plot of number of negative control features or background vs. net signal.

Red Signal Constant Term (C) Multiplier

The C term for the red channel is equal to this multiplier times the variance at the 25% percentile of a histogram plot of number of negative control features or background vs. variance.

Green Poissonian Noise Term (B) Multiplier

The B term for the green channel is equal to this multiplier times the net signal at the 25% percentile of a histogram plot of number of negative control features or background vs. net signal.

Green Signal Constant Term (C) Multiplier

The C term for the green channel is equal to this multiplier times the variance at the 25% percentile of a histogram plot of number of negative control features or background vs. variance.

CAUTION   The default values for these terms have been optimized for Agilent microarrays, with an Agilent scanner and recommended lab protocols. You must perform optimization experiments to determine the optimum values for your microarrays and lab protocols.

Parameters for a "False" setting

Figure 67   Flag Outliers — Agilent Scanner selected,
Automatically Compute OL Polynomial Terms: False

A noise model, based upon a polynomial equation, is made up of three variables whose values are shown in the Automatically Compute OL Polynomial Terms folder:

(%CV)^2 Term (A)   Same as for the "True" case above.

Poissonian Noise Term (B)   This term specifies the variance due to Poisson distributed noise, which accounts for error in counting statistics.

Background Term (C)   This term specifies the variance due to signal-independent background noise (e.g., scanner and glass substrate noise).

Changing Protocol Step 5: Compute Bkgd, Bias and Error

At this stage of the Feature Extraction process, several systematic sources may still remain that contribute to the background signal component of the feature signal:

• Scanner offset, which can vary from 20 counts to 300 counts, depending upon the version of Agilent scanner [B (20) or A (300) scanner]. The value for each microarray offset is shown in the Stats table in the fields: gDarkOffsetAverage and rDarkOffsetAverage (See the *Feature Extraction 10.10 Reference Guide.*)

• Glass and any contaminants or non-specifically-bound fluorescent signal on the glass. Artifacts from labeling, hybridization and washing steps can contribute to this source.

• Fluorescent signal that is non-specifically associated with the DNA probes themselves.

• A systematic gradient across a microarray.

Background region levels can also vary depending on the dye label and color channel used.

To access the settings to compute background, bias and error

- Click the **Compute Bkgd, Bias and Error** tab to select a method and change settings for background subtraction.
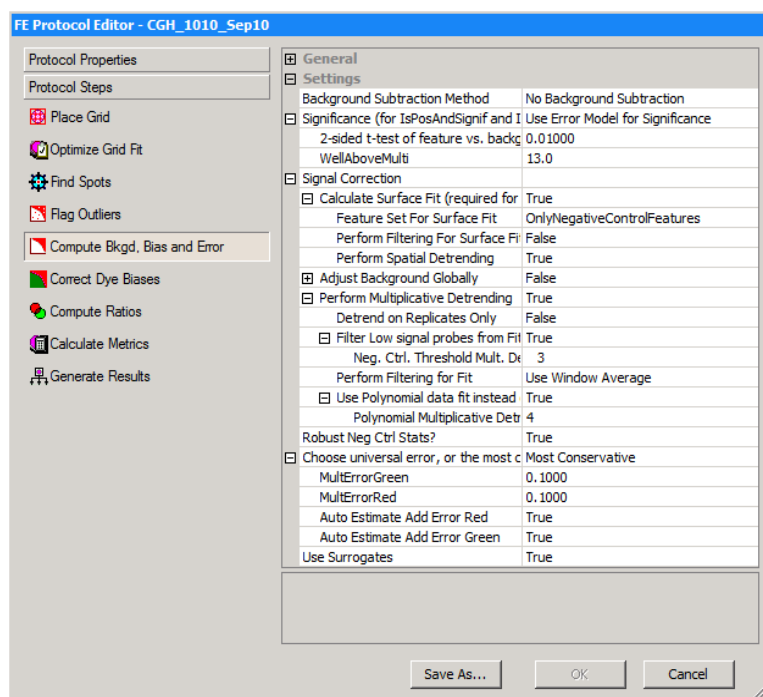
Figure 68   FE Protocol Editor — Correct Bkgd, Bias and Error

The selections for this protocol step let you decrease the contribution of the background signal to the feature signal.

- Select the background subtraction method.
- Choose to decrease the signal contribution due to an additive gradient at low signal across the microarray – spatial detrend.
- Choose to adjust the signal contribution due to a systematic multiplicative gradient across the microarray – multiplicative detrend. SNP probes are not included in multiplicative detrending.

To view the values used for background subtraction depending on the subtraction and correction methods selected, see the Feature Extraction 10.10 Reference Guide.

- Choose to globally adjust the initial background-subtracted signals.

- Choose an error method to estimate the error in the calculations for the processed signal.

- Change settings to assess if the background-subtracted signal is significant compared to the background.

- Choose to use or not use surrogates to improve the reliability of low-signal error calculations.

To select the background subtraction method

There are several methods for subtracting the background signal from the raw signal, both local and global, available in the Correct Bkgd and Signal Biases sheet.

1 Click the cell next to **Background Subtraction Method**, and click the down arrow.

2 Select the background subtraction method most relevant to your microarray and processing conditions.

The default method for Agilent microarrays is *No background subtraction*. The default method for non-Agilent microarrays loaded with a grid file is Minimum signal (feature) on the microarray. This option makes the fewest assumptions about the microarray system. If you are analyzing non-Agilent microarrays or procedures, you have to determine the optimum default method.

No Background Subtraction Even though no background is subtracted, you can make corrections to the signal using spatial detrend and the global background adjustment tools. The spatial detrend algorithm will correct for sources of background that are available in this protocol step, in addition to correcting for spatial gradients.

Local background method This method subtracts the local background estimated for each spot using the radius method in the Find Spots protocol step. For example, local background for spot 300 is subtracted from the feature raw signal of spot 300.

The local background method may be a better background subtraction method than a global background subtraction method if your microarray has large regions of local backgrounds with much higher signals than the rest of the microarray.

These regions are often due to hybridization or wash processing artifacts. The local background method subtracts these local variations. This yields a better estimate of true feature signal if the local background signal is correlated with the feature signal, as determined by replicate statistics.

Global methods   The Feature Extraction software supports a number of global background subtraction methods in case local background subtraction is not appropriate. With a global method, one number is calculated as background for the entire array. That number is subtracted from every feature. The number is reported in the STATS table for later queries. Global methods require that both non-uniformity outlier and population outlier methods be turned on. See the topic "Changing Protocol Step 4: Flag Outliers" on page 212 for more information.

Average of All Background Areas   This global method subtracts the average of the local background inliers estimated for all the spots using the radius method in the Find Spots protocol step. Inlier background regions have passed both the non-uniformity and population algorithms.

For example, the average background for all spots is subtracted from the feature raw signal of spot 300 and every other spot on that microarray.

Average of Negative Control Features   If the local background is not additive and your microarray has validated *negative controls*, Agilent recommends using the Average of negative control features option. If the local background is not additive and your microarray does not have negative controls, Agilent recommends the Minimum signal (feature) option.

Negative controls are probes that consistently show very little target binding across many types of targets and have no specific target bound to them by design.

This global method subtracts the average of the inliers of all the negative control features from each feature on the microarray. Inlier negative control features have passed both the non-uniformity and population algorithms. For example, the average of the inlier negative control features is subtracted from spot 300 and every other spot on that microarray.

Agilent provides validated negative controls for catalog microarrays. These probes have been shown to reflect the non-specific signal level that is present on probes.

If there is more than one probe sequence on the microarray annotated in the grid template or grid file as a negative control, this method analyzes the population of feature signals of each sequence to determine if there are significant differences among the different sequences at a fixed significance level of 0.01. The method then chooses the probe sequences that have the lowest mean signals and that are not significantly different from each other as the population of features to calculate negative control statistics.

Minimum signal (feature or background)

This global method subtracts the minimum signal on a microarray from each spot, whether the signal is a feature or local background region. The feature or background region signal chosen for the minimum signal must pass a low pixel standard deviation requirement. For example, the minimum feature or background signal on the microarray is subtracted from spot 300 and every other spot on that microarray.

Minimum signal (feature) on the array

This global method subtracts the minimum signal of a microarray feature that has passed a low pixel standard deviation requirement. This is the default setting for microarrays loaded with a grid file, but not necessarily the recommended setting. For example, the minimum feature signal on the microarray is subtracted from spot 300 and every other spot on that microarray.

To choose to remove the surface trend found in the data

What is detrending?

To learn more about the detrending calculations, see the Feature Extraction 10.10 Reference Guide.

Removing the surface trend (detrending) decreases the contribution to the background of any systematic signal gradient on the microarray. For both 2-color and 1-color data, Feature Extraction removes the additive surface trend from the data, a process called *spatial detrending* (background detrending). Feature Extraction also removes any "domed" surface trend, a process called *multiplicative detrending*. SNP probes are not included in multiplicative detrending calculations.

This option is the default choice with protocols for Agilent microarrays (except the miRNA protocol). The detrending is done individually for each color channel and corrected separately.

Background detrending consists of two steps: calculating a surface fit and subtracting the surface fit from the data. This process is done independently for the red and green channels after background subtraction and before the global background adjustment.

Background Spatial Detrending

For spatial detrending, Agilent separates the surface fit and subtraction calculations that must exist together to remove the surface trend found in the data.

The *foreground* is the portion of the signal in a feature that is not related to the intended signal from the dye-labeled target complementary to the probes on that feature.

The surface fit calculation estimates the "foreground" of the data by measuring the intensity of the feature after background subtraction (or intensity of the MeanSignal of the feature if the "No background subtraction" option is selected) and performs a surface fit to the selected data. Even if the surface fit is not subtracted from the data (perform spatial detrending), the calculation appears in the QC Report to help evaluate your data.

The advantage of this method is that it can remove variation in foreground intensity for different regions of the microarray. By performing spatial detrending and subtracting the surface fit through this foreground, the data becomes more consistent and reproducible.

Feature Set for Surface Fit

AllFeatureTypes   Select if microarray has a set of very low intensity features evenly distributed on the slide.

OnlyNegativeControlFeatures   Select this if you have enough negative controls that are evenly spread around the microarray and ideally have different sequences represented – default for CGH and ChIP protocols.

FeaturesInNegativeControlRange   Select for low intensity features that are within 3 standard deviations of the spatial interpolated values of the negative controls - default for gene expression and miRNA protocols.

Perform Filtering for Surface Fit

If True, allows a subset of the features into the set to be used to calculate the surface fits. You may want to do this to reduce the time needed to calculate the surface without reducing the accuracy of the fit.

If the Feature Set is AllFeatureTypes, then the filter chooses 1 to 2 percent of the dimmest features to be selected across the array. Select True for AllFeatureTypes. Select False for OnlyNegativeControlFeatures.

If the feature Set is FeaturesInNegativeControlRange, the filter chooses 1 to 2 percent of the features at random to go into the surface fitting set. Random x% rather than dimmest x% is used because the features in this set are already known to be dim.

Perform Spatial Detrending

If true, subtracts any additive trend found from the surface fit from the data.

To choose to adjust the background globally (2-color)

To correct the errors at the low end of the signal range, Agilent lets you choose to globally adjust the background after initial background subtraction. The global adjust method was developed to be used by those customers who decide to turn off all background and detrending methods.

This adjustment helps estimate and remove background signal due to fluorescent signal that is non-specifically associated with the DNA probes and that may be under-estimated by upstream background subtraction methods. You can also select this correction if you also chose *No background subtraction*.

1 Click the cell next to **Adjust Background Globally**, and click the down arrow.

2 Select **True** or **False**.

3 If you select True, enter a "pad" value between 0 and 500 to force the log ratio towards zero when both red and green signals are small.

The constant supplied to the *Adjust background globally* algorithm "pads" all the feature signals by that value. This will have the effect of compressing log ratios, but will decrease the variability (standard deviation) in the log ratio among inter- and intra-array replicates. You can try small pad values, typically in the few tens, and monitor the trade-off between log ratio compression and log ratio variability. The log ratio versus signal plot is a useful preliminary tool to assess the effect of the pad value.

By setting the *Adjust background globally* algorithm to the same constant with multiple microarrays, the residual background can now be defined by you and is therefore more consistent across multiple microarrays and multiple experiments than if a variable residual background signal is left for each feature, if no background is subtracted.

To choose to perform multiplicative detrending

Multiplicative detrending is an algorithm designed to compensate for slight linear variations in intensity if the processing is not homogeneous across the slide, resulting in different chemical reaction concentrations, for example, between the sides and the center, a "dome" effect. The multiplicative gradient is removed by dividing the signal by the observed gradient. SNP probes are not included in multiplicative detrending calculations.

• Make the selections described below.

Perform Multiplicative Detrending   Choose whether to perform multiplicative detrending (can be chosen for one- or 2-color arrays).

Detrend on Replicates Only   Choose whether to detrend on all of the data or to only use those probes that have replicates. If you choose to use replicates only, the data are normalized to the respective replicate averages before going into the fitting set.

Filter Low signal probes from Fit?   You can chose to filter out dim signals from the fit of the multiplicative trend surface. This is useful because the dimmer signals have a less obvious trend when that trend is multiplicative. You also choose how dim the signals must be in order to be removed from the set. This is stated as a multiple of the Negative Control Spread.

Perform filtering for fit   You must decide whether to use all of the data points or use local averages or medians.

To choose to use robust negative control statistics

This algorithm was developed to be used with microarrays that have many different probes classified as negative controls, such as CGH. The population outlier analysis, described on the previous pages, is performed on these negative controls, one probe sequence at a time.

- Select a **Robust Neg Ctrl Stats?** option.

True Performs a second round of population outlier analysis on the entire population of negative control features

Once outliers are identified, they are excluded from the calculation of the robust negative control statistics (average and standard deviation).

False Does not perform a second round of population outlier analysis

See the *Feature Extraction 10.10 Reference Guide* for more information on how this algorithm works.

To select the method to estimate the error (Error Model)

1 Click the cell next to **Choose universal error, or the most conservative (propagated or universal)**, and click the down arrow**.**

2 Select an error method from one of these options:

Universal Error The Universal Error Model represents the expected error of the difference between the red channel and the green channel. This error is represented as the square root of the sum of two squared components from each channel: a constant noise term (**additive error**) for each channel, and an intensity scaled term (**multiplicative error**).

Using this error model, a p-value is calculated that corresponds to the probability that the difference between the red and green channels is zero. The Universal Error Model is a good estimator of error in high intensity features.

It may underestimate the error of low signal features or features that have low signal-to-noise (e.g., non-uniform features).

Most Conservative This is the recommended method. This is a hybrid method which evaluates both the propagated error model and the Universal Error Model, and reports the higher p-value, and therefore the more conservative estimate of error, of the two models. This method captures the advantages that the propagated error model has for low intensity features and features with low signal to noise and the advantages the Universal Error Model has for high intensity features.

Propagated Error This method measures the error on the log ratio by propagating the pixel-level error of that feature through all the calculations on the raw signal, including raw pixel statistics, background subtraction, dye normalization and the log ratio calculation. Agilent follows standard propagation of error techniques and calculates a p-value that corresponds to the probability that the log ratio = 0.

The propagated error is a good representation of noise that predominates at low signal ranges, such as instrumentation noise and the noise due to non-uniform features.

The propagated error cannot capture other sources of noise that dominate at higher signal ranges, such as biological and chemical variability (e.g., writing and labeling, hybridization and wash processes). The propagated error method usually underestimates the noise, especially at higher signal levels.

See "Parameters for Universal and Most Conservative error models" on page 229 for more instructions on changing the parameters for these two error models.

Parameters for Universal and Most Conservative error models

Parameters to change With this error model and the most conservative error model, you can enter a change to the following settings and parameters:

MultErrorGreen (Red) Enter the multiplicative error component in the channel selected

Autoestimate Additive Error Green(Red)   You can select to enter your own constant for the additive error or turn on auto-estimation of the additive portion of the error model. If you select **True**, then Feature Extraction will auto-estimate the additive error. If you select **False**, then you must enter the constant.

The constant setting of the additive error approximates the error of feature signals that are near background. If the overall signal of the microarrays is increased or decreased (e.g. due to "hotter" or "cooler" labeling or variable scanner PMT settings), the additive error needs to be adjusted. The auto-estimation method of determining the additive error adjusts the scale accordingly.

CAUTION   The default additive and multiplicative parameters have been optimized using the most current Agilent microarray-based system (e.g., microarrays, protocols, scanner and most recent Feature Extraction algorithms). If you use non-Agilent microarrays or Agilent microarrays with non-Agilent protocols, you MUST re-evaluate these values to reflect the noise in your system.

Auto-estimation of additive noise   Provided that the microarray, platform and protocols remain the same, the additive error can vary from one microarray to the next. Therefore, it is useful to correctly estimate the additive noise and set the additive constant equal to this noise.

To do this the software looks at microarray statistics: the width of the negative control distribution used in the background surface fit and the spatial variability of the spatial detrend surface (RMS of the difference between each point on the surface and the mean of the surface). Because the algorithm uses these surface statistics in the calculation, this option is only usable for microarrays for which surface calculation works.

You do not have to turn on "Spatial detrending" for the additive error to be automatically estimated. You do have to turn on the surface fit calculation for the additive error to be estimated.

Reliability of auto-estimation of additive error   Agilent has found that the auto-estimation of the additive error results in error values that are more reliable than those generated if a constant is used. If the microarray quality or processing is less than optimal, the additive error is more reliable with auto-estimation. This will make the significance calls more correct and less susceptible to errors induced by microarray and processing variability.

To choose to use or not use surrogates

Agilent Feature Extraction software uses surrogate signals to calculate a more accurate and reproducible log ratio for signals of low intensity.

You choose to use surrogates in this protocol step so that the signals to be used for log ratios are subjected to dye normalization to remove this kind of bias.

Agilent Feature Extraction software uses the error estimate option that you select for all features, no matter if surrogates are used for the log ratio calculations for some features.

1 Click the cell next to **Use Surrogates**, and click the down arrow.

2 Select **True** or **False**.

Conditions under which surrogates are used

If you select True, the software uses surrogates under two conditions:

• If either red or green MeanSignal fails the IsPosAndSignif test (i.e., IsPosAndSignif = 0)

• If BGSubSignal is less than the Error

A log ratio cannot be calculated from a negative ratio. For any measurement system there is increased confidence in using a signal only if it is greater than a lower limit that is particular to that system. This ensures that the system lower limit is enforced as a lower limit on the signal.

Feature Extraction calculates the surrogate signal from the Error. For all default Agilent protocols that use Error Model significance, this is simply the Additive Error calculated during background subtraction.

Propagation of errors from the Compute Bkgd, Bias and Error algorithm to other algorithms

The measurement errors from the background subtraction are propagated to the next steps in Feature Extraction according to standard error propagation techniques. For example, the errors for the feature and background signals are propagated with the error on the background-subtracted signal to the DyeNorm algorithm.

Estimation of error is performed in this step. See "To select the method to estimate the error (Error Model)" on page 228 to see the choices show for this step. In this case, you cannot select the propagated error calculation by itself. If you select Most Conservative, Feature Extraction uses the larger of the two estimates from the Universal Error model or the propagated error.

To change settings to test the significance of a feature

Determining if a signal is significant, or just in the noise of the background, is an important step. The following two tests are performed to establish the significance of a feature: a two-sided t-test and a method to flag features that are "well above" background.

2-sided t-test of feature vs. background maximum *p*-value

The t-statistic is calculated based on the feature and background signals and their corresponding errors.

The background value used for this test depends upon the background method used. The values are reported in the Feature Extraction text output column "g(r)BGUsed". If you have chosen not to subtract the background and Spatial detrend = Off, the local background for each feature {g(r)BGMeanSignal} is used for the t-test but is not subtracted from the feature signal. If Spatial detrend = On when the background subtraction is off, the surface value for each feature is used for the t-test and is subtracted from the feature signal.

To view the values used for the t-test depending on the subtraction and correction methods selected, see the Feature Extraction 10.10 Reference Guide.

Estimating background error for significance testing uses one of two methods. The method used is dependent upon the protocol parameter, "Significance (for IsPosAndSignif and IsWellAboveBG)".

Pixel significance

For this method, the SD of the background that is used for the t-test is the same value used for surrogates (See "Use of surrogates for log ratio and p-value calculations" on page 243 of this guide). This is a population level SD for global background methods and is a pixel-level SD for both the local background and No background methods. These SD's are reported in the Feature Extraction text output columns "g(r)BGSDUsed". (Not used for error model significance, which is the favored method.)

The number of degrees of freedom is also based on the error calculations and the number of pixels, which depends on the background subtraction method. The p-value is then calculated based on a two-sided t-test. If this p-value is less than the maximum p-value defined in the protocol, the feature is considered to be significantly different from the background.

Note that entering a positive pad value for global background adjust may increase the number of features considered positive and significant in the t-test, in some cases, all features.

The features that "fail the null hypothesis", that is, have a signal that is significantly different from the background and also that have background-subtracted signals > 0 will have a boolean set = 1 in the Feature Extraction text output column "g(r)IsPosAndSignif". The features that are not significantly different from background or that have background-subtracted signals < 0 will have the boolean set = 0 for the same Feature Extraction text output column.

WellAboveMulti Features that have background-subtracted signals that are greater than "WellAboveMulti*BackgroundSignal Standard Deviation" are flagged as well above background. A default value of 2.6 SD for the multiplier is consistent with a 99% confidence interval. The text output for the result of this test appears as g(r)IsWellAboveBG in the Features table of the output text file.

Error model significance

The other method to determine feature significance is based on the error model. The protocol parameter "Significance (for IsPosAndSignif and IsWellAboveBG)" is set to "Use Error Model for Significance" for this method. The additive error is used as the estimate of background noise and is used for surrogates.

The significance test for positive and significant then becomes the probability of the feature actually having zero signal for a feature with a given background-subtracted signal with a known additive error (or noise of features with no true signal) assumed to be gaussian distributed about zero.

You choose a p-value to decide how far along the Gaussian distribution the feature needs to be before it should be considered Significant. When this choice is made, the Well Above Background is decided by a multiple of the additive error.

Changing Protocol Step 6: Correct Dye Biases

A *dye bias* is the difference in the red and green signals caused by different efficiencies in labeling, emission or detection.

Systematic differences in dye bias must be removed from the 2-color GE, CGH, and ChIP data. To remove dye bias, the Feature Extraction software uses a dye normalization algorithm. This step is not used with GE 1-color and miRNA data.

To access the settings to correct dye biases

- Click the **Correct Dye Biases** button to change settings to remove difference in dye bias.
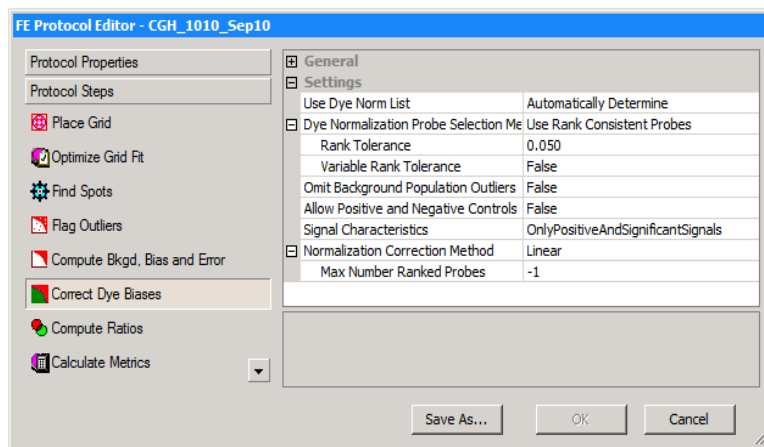
Figure 69   FE Protocol Editor — Correct for Dye Biases selected

You can change the following types of settings for this algorithm.

- Select how dye normalization gene list is determined
- Select features that are included in the normalization set
- Select to omit background population outliers
- Select to allow positive and negative controls

- Select the signal characteristics

- Select a curve to determine the bias from that normalization set of features

  The software normalizes the entire microarray from this measured bias.

If you use a self versus self experiment, in which the feature signals should be the same in both channels across the dynamic range of signal intensities, differences in dye behavior can result in the signals in one channel increasing across the signal range at a different rate than in the other channel.

This behavior can be visualized by plotting the background-subtracted signals of one channel versus the other and noting that the slope of the line fit through the data points is not equal to one. Dye normalization corrects for this by multiplying the signals by appropriate dye normalization factors.

To choose how to select dye normalization list

Three options are available for choice of the dye normalization gene list for correction of dye biases.

1 Click the cell next to **Use Dye Normalization Gene List**, and click the down arrow.

2 Select one of the following choices.

Automatically Determine   The DyeNormList is used, if present in the grid template. Otherwise, the list is generated internally, based on the Dye Normalization Probe Selection Method. If no DyeNormList is selected in the grid template, extraction will not fail.

True   When this is selected, the DyeNormList from the grid template is used. Extraction fails if there is no DyeNormList selected in the grid template.

False   When selected, a DyeNormList will not be used even if it is specified in the grid template. During the dye normalization step, an internal dye normalization list is generated, based on the Dye Normalization Probe Selection Method.

To choose a method to select a set of dye normalization probes

Feature Extraction supplies two methods for selecting representative features that constitute the set used for bias measurement.

1 Click the cell next to **Dye Normalization Probe Selection Method**, and click the down arrow.

2 Select one of the following methods.

Use Rank Consistent Probes   This is the recommended method of selecting normalization features. The rank consistency filter selects features that fall within the central tendency of the data by observing consistent trends between the red and green channels. This method assumes that the central tendency of the data is unregulated. This method requires that the features pass the three criteria described below:

• They have not been flagged as non-uniform in either channel.

• They are not saturated.

   The condition for saturation is more stringent in this algorithm (> 5% of pixels saturated) than in the algorithm for features as saturated (> 50% of pixels saturated).

• They are not population outliers in either channel.

Rank Tolerance   You can enter a *Rank Tolerance* value if you select the Use Rank Consistent Probes choice. The *rank tolerance* is the red rank value minus the green rank value divided by the number of features, considered for rank consistency, times 100, and expressed as a percentage. If this value is 5, for example, then a rank consistent feature would be one whose red rank did not vary above or below the green rank by more than 5%.

To assign a red rank and a green rank, order all of the probes based on the intensity of their background-subtracted signal. A rank 1 is the lowest intensity signal feature. The highest rank is assigned to the most intense signal. A perfectly "rank consistent" feature would be one exactly at the 75% percentile of the distribution in both the red and green signal.

Variable Rank Tolerance   You can choose to allow variable rank tolerance. This does rank tolerance after grouping the data into signal groups. In each group the same designated percentage of most rank-consistent data are included.

Use All Probes   This method assumes that the average log ratio over all inlier non-control features should be zero. All non-control features that pass the four criteria listed for the Rank Consistent Probe method are candidates for this method set.

To select to omit background population outliers

1 Click the cell next to **Omit Background Population Outliers**, and click the down arrow.

2 Select **True** or **False**.

Selecting True excludes any features from the dye normalization set if the local backgrounds associated with those features have been flagged as population outliers (in either channel). The default recommendation is False.

To select to allow positive and negative controls

If you turn this function on, Feature Extraction uses positive and negative controls in the dye normalization calculation.

1 Click the cell next to **Allow Positive and Negative Controls?**, and click the down arrow.

2 Select **True** or **False**.

The default recommendation is **False**.

To select signal characteristics

You can select to include features with the following signal characteristics as part of the set of rank consistent features.

1 Click the cell next to **Signal Characteristics**, and click the down arrow.

2 Select one of the following options.

OnlyPositiveandSignificant Signals   Use only positive and significant signals for ranking in the rank consistency filter.

AllPositiveSignals   Use all positive signals for ranking in the rank consistency filter.

AllNegativeAndPositiveSignals   Use all negative and positive signals for ranking in the rank consistency filter.

To select a normalization correction method

After you select a set of normalization features, you select a normalization method to apply to that set. The results from the calculation are applied to all features on the microarray.

1 Click the cell next to **Normalization Correction Method**, and click the down arrow.

2 Select from one of the following methods:

Max Number of Ranked Probes   The maximum number of ranked probes allows only a certain number of probes into the dye normalization set. If the number of rank-consistent probes exceed this number, a random set is chosen to meet the maximum number. If the number entered is -1, this filter is not used. This option can speed up the data processing without adversely affecting accuracy for data sets with large numbers of probes, especially for 65-micron spot size and higher density arrays.

Linear   This method multiplies the background-subtracted signal by a global constant. The global constant is determined separately for the red channel and the green channel using the selected normalization features. It is calculated using the geometric mean of these features as a value of 1000.

Linear and LOWESS   This is the default method for normalizing Agilent microarrays. Dye biases tend to be signal intensity-dependent. A dye normalization method that uses a constant scaling factor cannot correct for dye biases across the entire range of signal intensities. This method does a linear normalization across the entire range of data, then applies a non-linear normalization (see LOWESS only description below) to the linearized data set.

LOWESS only   This method assumes the dye bias is intensity-dependent, and therefore the background-subtracted signal is multiplied by a value that is a function of the feature intensity. The selected normalization features are used to calculate a normalization curve that measures the potential log ratio bias across the entire range of feature intensities.

A well-known curve fitting method called LOWESS (a locally weighted linear regression curve fit) is used to fit this curve. Given any feature and its intensity, this curve determines the potential dye bias that should be present in the log ratio measurement of the feature. Features are then corrected by multiplying the red and green channels by an adjustment that will remove the bias from the log ratio.

Changing Protocol Step 7: Compute Ratios

Log ratio is the log (base 10) of the ratio of the red channel signal to the green channel signal.

Log ratio error is the error about the mean of the log ratio.

P-value is a measure of confidence that a feature is, or is not, differentially expressed.

To determine if a feature is differentially expressed, three measures are important: *log ratio*, *log ratio error* and the *p-value*.

The log ratio error is determined by the error model and therefore used to help assess the significance of the computed log ratio.

The p-value is calculated using the log ratio and the log ratio error. Genes with lower p-values have higher confidence of being differentially expressed. For example, if the p-value is <= 0.01, there is a 99% confidence level that the gene is differentially expressed.

This step is not used with GE 1-color and miRNA data.

To access the settings to compute ratios

*   Click the **Compute Ratios** tab in the FE Protocol Editor to change the settings to calculate these measures.

    See Figure 70 on page 243.
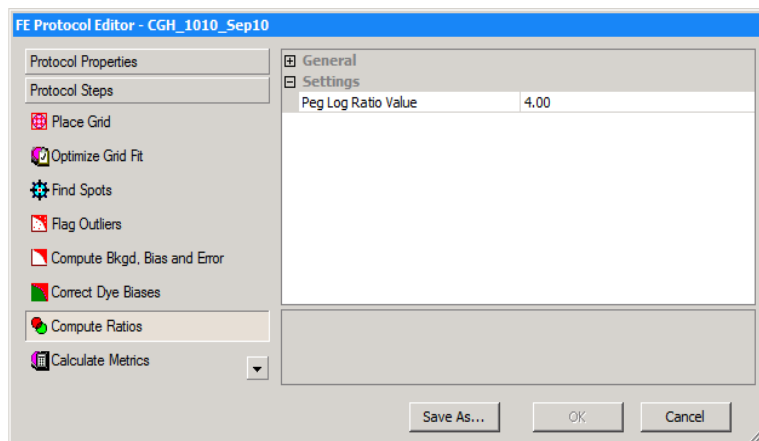
Figure 70   FE Protocol Editor — Compute Ratios selected

You can change only one parameter for this protocol step:

• Enter the value for the log ratio limit.

To enter the value for the log ratio limit

When you set a limit, the features whose log ratio exceeds that limit, either in a positive or negative direction, will not be used in any calculations. The recommended limit for this value is 4.

1 Click the down arrow in cell next to **Peg Log Ratio Value**.

2 Enter a positive or negative value up to 4.

Use of surrogates for log ratio and *p*-value calculations

Surrogates are used in this step to calculate certain log ratios, *p*-values and log ratio errors.

The table below shows the conditions for use of surrogates to calculate log ratios, log ratio errors and *p*-values.

Table 9   Use of surrogates for log ratio and *p*-value calculations

If red channel signal is	And green channel signal is	Then ratio is	And Feature Extraction does this*.
Positive and significant vs. background and greater than red background SD (R)	Positive and significant vs. background and greater than green background SD (G)	**R/G**	Uses the dye-normalized signals for both channels for the calculations.
Not positive and significant vs. background or less than red background SD (r)	Positive and significant vs. background and greater than green background SD (G)	**r/G**	Uses the dye-normalized green signal and the dye-normalized red background SD signal (surrogate) for the calculations.
Not positive and significant vs. background or less than red background SD (r)	Not positive and significant vs. background or less than green background SD (g)	**r/g**	Uses surrogates for both channels. Sets the log ratio = 0. Sets the *p*-value = 1.
Positive and significant vs. background and greater than red background SD (R)	Not positive and significant vs. background or less than green background SD (g)	**R/g**	Uses the dye-normalized red signal and the dye-normalized green background SD signal (surrogate) for the calculations.

*For log ratios equal to or between -4 (ratio = .0001) and +4 (ratio = 10000). If (r/G) > 1 or (R/g) < 1, log ratio = 0 and *p*-value = 1.

Table 10   Summary – Use of surrogates for calculations

Case 1: R/G Both channels use DyeNorm Signals. *P*-value and log ratio are calculated as usual. For signals not using surrogates, g(r)DyeNormSignal = g(r)ProcessedSignal, which is then used to calculate log ratio.	Case 2: r/G r = rSurrogateUsed G = gDyeNormSignal *P*-value and log ratio are calculated as usual. If r/G > 1, then Feature Extraction automatically sets LogRatio = 0 and PvalueLogRatio = 1
Case 3: R/g R = DyeNormSignal g = gSurrogateUsed *P*-value and log ratio are calculated as usual. If R/g < 1, then Feature Extraction automatically sets LogRatio = 0 and pValueLogRatio = 1	Case 4: r/g Both channels use surrogates. Feature Extraction automatically sets LogRatio = 0 and pValueLogRatio = 1 For signals using surrogates, g(r)ProcessedSignal = g(r)SurrogateUsed x g(r)DyeNormFactors.

Effect of using surrogates on log ratios

Below are plots that show the difference between using surrogates and not using surrogates for a set of self vs. self microarray data.
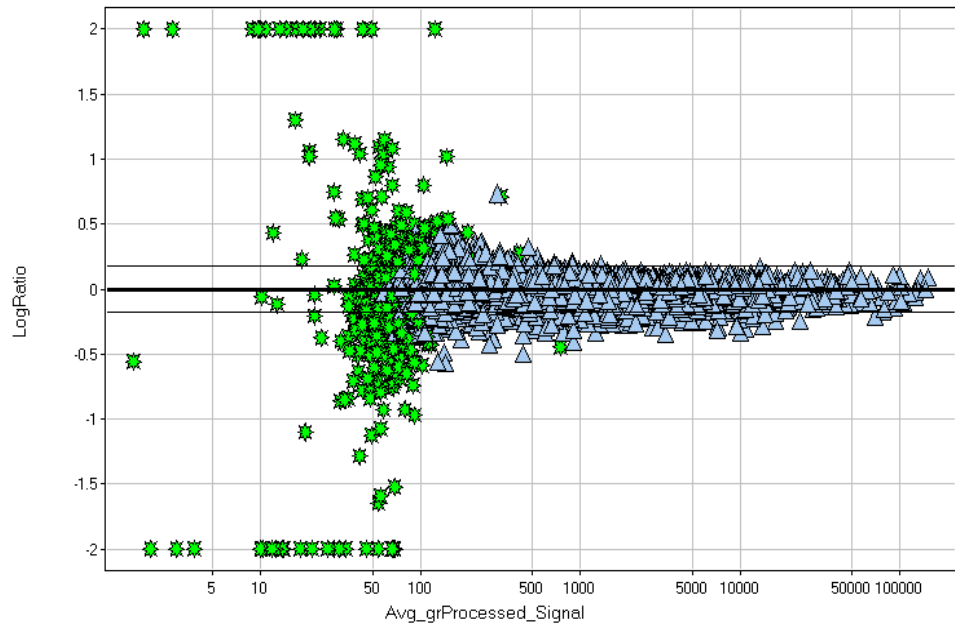
Figure 71   Plot of LogRatio vs. Avg_grProcessedSignal–No surrogates

Green stars are data points affected by use of surrogates in Figure 72.
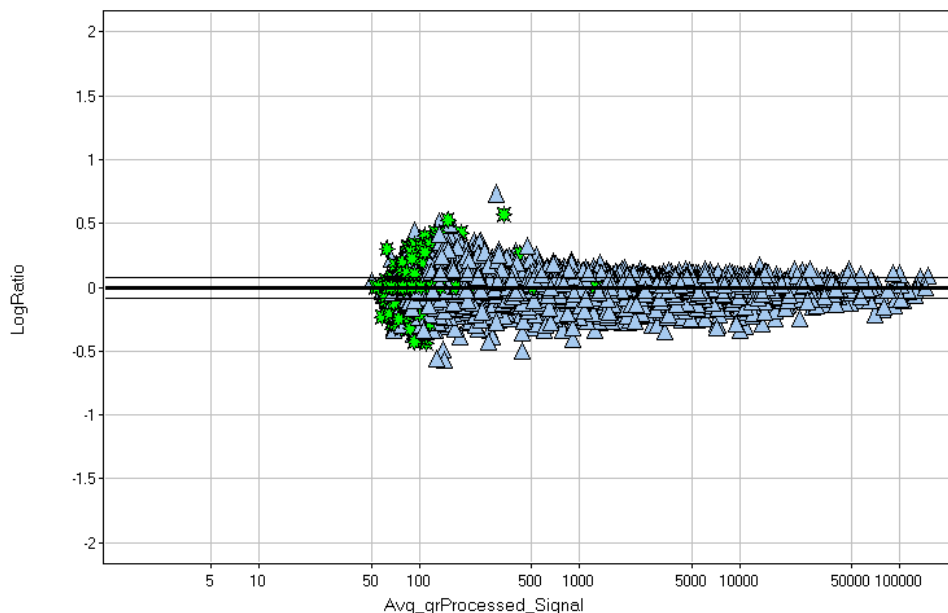
Figure 72   Plot of LogRatio vs. Avg_grProcessedSignal–With surrogates

Green stars are data points affected by use of surrogates. See Figure 71 for their positions without surrogates.

Effect of using surrogates on log ratio errors

If "Use Surrogates" is True, the software calculates log ratio errors using the dye-normalized and/or surrogate signals, according to the error model selected for the calculation.

If "Use Surrogates" is False, the software calculates log ratio errors using the dye-normalized signals, according to the error model selected for the calculations. If either or both channels have negative dye-normalized signals, the p-value is set to 1 and the log ratio error is set to 1000. If red channel < 0, then Feature Extraction sets the log ratio = -4. If green channel < 0, then Feature Extraction sets the log ratio = +4. If both red and green channels < 0, then Feature Extraction sets the log ratio = 0.

Changing Protocol Step 8: MicroRNA Analysis

This step appears only in the miRNA protocol. With this step you set up to calculate a Total Gene Signal. See the *Feature Extraction 10.10 Reference Guide* for more details on how Feature Extraction processes a miRNA image and on the QC report and text file results, algorithms and protocol default values.

Both the protocol parameters and default values may change over time. To ensure that you have the latest protocols, you must set up eArray login and let Feature Extraction automatically check the eArray server for the protocol updates every time the software starts. See "Setting up eArray login for automatic updates" on page 36.

To access the settings to analyze microRNA (miRNA) images

• Click the **MicroRNA Analysis** tab in FE Protocol Editor to change the settings for miRNA analysis.
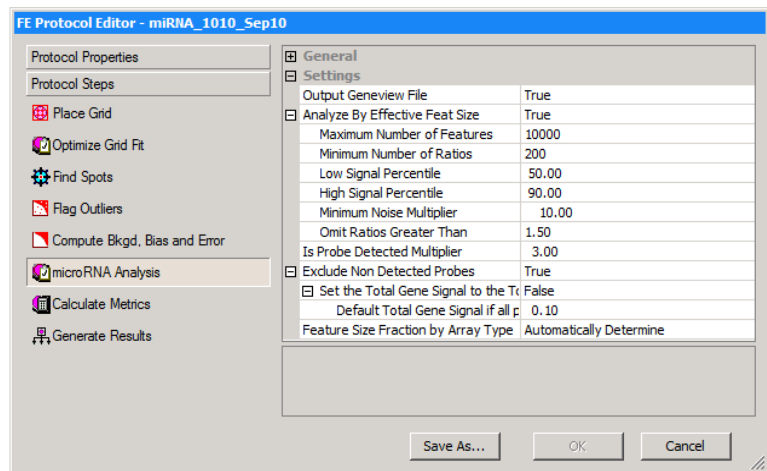
Figure 73   FE Protocol Editor – miRNA protocol — microRNA Analysis selected

You can change the following settings for this protocol step.

- Choose to generate the results to a GeneView file, or not.
- Choose to scale the analysis with the effective feature size parameters, or not.
- Enter an Is Probe Detected multiplier.
- Choose to exclude the non detected probes, or not.
- Choose to automatically determine feature size fraction or by array type.

To choose to generate the results to a GeneView file

After running Feature Extraction with the microRNA protocol, a new file is generated in addition to the tab-text file, MAGE-ML file, QC Report and any other files selected. This file is saved in the same directory as the other result files and is named ArrayName_GeneView.txt.

Each row includes a "gene" on the microarray, or a unique Systematic/Gene name in the design. This file is a tab-text file with the following columns:

- SystematicName
- ControlType
- gTotalGeneSignal
- gTotalGeneError
- IsGeneDetected

1 Click the cell to the right of **Output GeneView File**.

2 Click the down arrow, and select **True** or **False**.

To choose to scale the analysis with the effective feature size parameters

The calculation of the feature size is different for miRNA images. Even though you can change the default value from True to False, you will almost never have to do this.

1 Click the cell to the right of **Analyze By Effective Feat Size**.

2 Click the down arrow, and select **True** or **False**.

Even though the default value is True and these parameters appear, you will almost never have to change them:

Maximum Number of Features Maximum number of features used to compute the effective size.

Minimum Number of Ratios Minimum number of ratios used to compute the effective size.

Low signal Percentile Signal filter for computing the effective size of the spot. Uses signal in at least n (default–50%) percentile of all signal.

High Signal Percentile Signal filter for computing the effective size of the spot. Uses signal in at most n (default–90%) percentile of all signals.

Minimum Noise Multiplier Makes sure the signals are at least x times above the background noise for use in computing the effective spot size.

Throw Away Ratios Greater Than Ratios > 1 mean the outside of the cookie is brighter than the inside of the cookie. This may be due to crescents or non-uniformity or other anomalies.

To change the Is Probe Detected multiplier

This determines the Is Probe Detected multiplier in the miRNA algorithm.

1 Click the cell next to **Is Probe Detected Multiplier**.

2 Enter the desired value from 1.0 to 20.0. (The default value is 3.00.)

To choose to exclude non detected probes

If this option is set to False, the results will be the same as in Feature Extraction 10.5.

This changes how the TotalGeneSignal is calculated. If a TotalProbeSignal is not detected, then it is not added to the Total Gene Signal. In the miRNA analysis, the features relating to each probe is first turned into Total Probe Signals. The TotalProbeSignals for each probe associated with the target miRNA (Gene) are then summed. If a probe associated with an miRNA is not detected because it fails the IsProbeDetected flag, then if this option is True, it will not contribute to the TotalGeneSignal, and its error will not propagate to the TotalGeneError.

Set the Total Gene Signal to the Total Signal Error

By default this is set to False. When this option is set to True, the Default Total Gene Signal if all genes are not detected option is not used. This option is used only if all the probes in an miRNA (Gene) are not detected and the non detected probes are excluded from the calculation (Exclude Non Detected Probes set to True.) If this option is set to True, the value of the TotalGeneError if all probes were included is used for the TotalGeneSignal. Another option appears that allows the user to set a fixed value for the TotalGeneSignal if no probes are detected. That value by default is 0.10.

Default Total Gene Signal if all probes are not detected

Used only if all the probes in an miRNA are not detected, and the non detected probes are excluded from the calculation (Exclude Non Detected Probes set to True.) For an miRNA where all of its probes are not detected, then the TotalGeneSignal is set to this value to avoid errors caused by the algorithm trying to calculate a log of 0.

Changing Protocol Step 9: Calculate Metrics

In this step you can change the settings for the metrics that appear in the QC report.

To access the settings to calculate metrics

• Click the **Calculate Metrics** tab in the FE Protocol Editor.
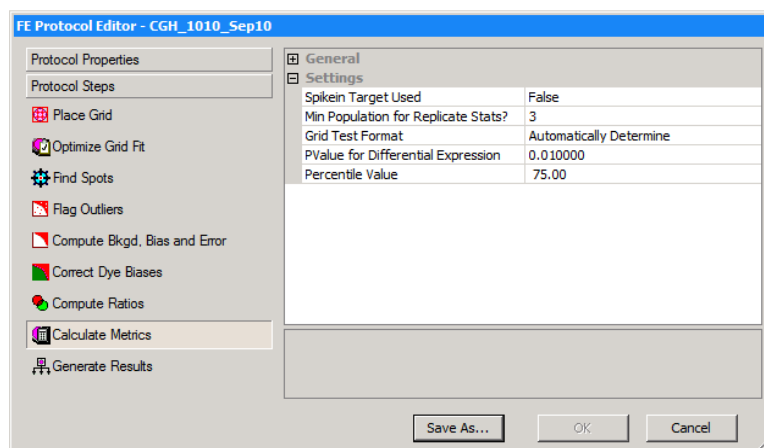
Figure 74   FE Protocol Editor — Calculate Metrics selected

You can change the following parameters in this step:

• Select True for Spikein Target Used if Agilent spike-in targets were used during lab processing of the microarray. The appropriate metrics are generated in the QC report.

• Enter the minimum population for replicate statistics.

• Select the grid test format.

• Enter the p-value for differential expression.

• Enter the percentile value.

To select to use spikein targets

1 Click the cell next to **Spikein Target Used?**, and click the down arrow.

2 Select **True** or **False**.

If you select True, Feature Extraction will do all the calculations necessary to report the statistics for both the non-control features and the spikein controls.

To change the minimum population for replicate statistics

The QC report includes statistics based on replicate probes (%CV, log ratio uniformity). Feature Extraction must know the minimum population of replicates in order to calculate these statistics.

1 Click the cell next to **Minimum Population for Replicate Statistics**.

2 Enter a number of 3 or greater.

To select the grid test format

1 Click the cell next to **Grid Test Format.**

2 Select the grid format of the images to be tested, or select **Automatically Determine** to let the software decide for you.

To change the *p*-value for differential expression

The QC report contains a plot of the log ratio vs. the magnitude for each feature to show which features are up-regulated, which are down-regulated and which show no differential expression. This assessment is relative to the *p*-value chosen for this setting.

1 Click the cell next to **PValue for Differential Expression**.

2 Enter a *p*-value against which you intend to measure differential expression on the microarray.

To change the percentile value

This value is an indicator of the distribution of signals of the non-control features and is useful in downstream normalization of 1-color data.

1 Click the cell next to **Percentile Value**.

2 Enter a value to set the distribution of signals of the non-control features.

Changing Protocol Step10: Generate Results

To access the settings to generate results

- Click the **Generate Results** tab in FE Protocol Editor to change the values for output.
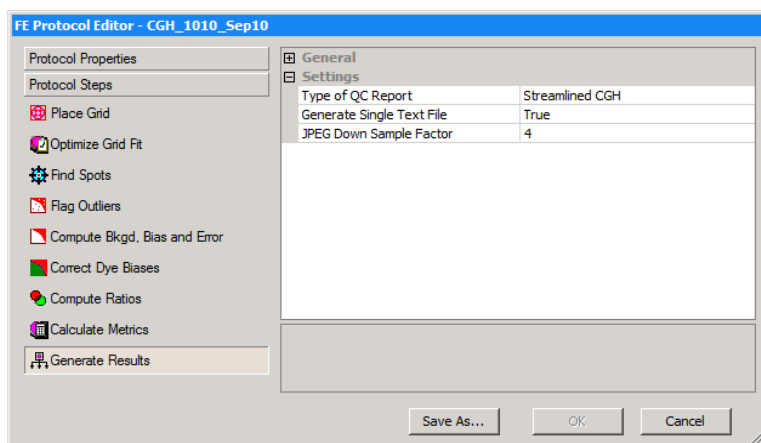
Figure 75   FE Protocol Editor — Generate Results selected

You can change the following settings for this protocol step:

- Select the QC Report type.
- Select to generate a single file for the text output.
- Change the compression factor for the JPEG output.

To select the type of QC Report

1 Click the cell next to **Type of QC Report**, and click the down arrow.

2 From the list, select the QC report type to be generated.

Gene Expression   Generate a Gene Expression QC report. For more information, see the *Feature Extraction 10.10 Reference Guide*.

Streamlined CGH   Generate a Streamlined CGH QC report. For more information, see the *Feature Extraction 10.10 Reference Guide*.

CGH_ChIP   Generate a standard CGH_ChIP QC report. For more information, see the *Feature Extraction 10.10 Reference Guide*.

miRNA   Generate an miRNA QC report. For more information, see the *Feature Extraction 10.10 Reference Guide*.

Test Chip   Generate a Test Chip report. This report is used by Agilent service personnel for scanner performance verification.

To select to generate a single file for the text output

1 Click the cell next to **Generate Single Text File?**, and click the down arrow.

2 Select **True** or **False**.

If you select true, the text output appears in a single file. If you select False, it appears in three separate files, one for FEPARAMS, one for STATS and one for FEATURES (results).

To change the compression factor for the JPEG output

The value for this factor determines the degree to which the image is scaled down in both directions before being converted to JPEG.

1 Click the cell next to **JPEG Down Sample Factor**, and click the down arrow.

2 Enter a number.

4 Changing Protocol Settings

To change the compression factor for the JPEG output

5
Changing Image Displays

This chapter presents instructions on changing image displays and working with image files to view the images before and after extraction.
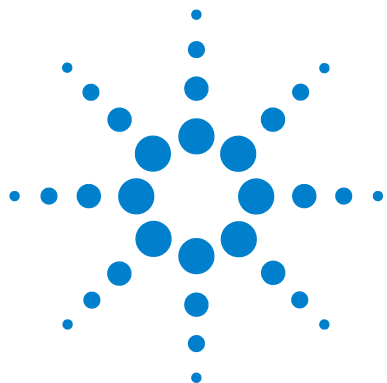
Agilent Technologies

Displaying and saving image files

To open an image file

1 Click on the toolbar **Open image file** button, or click **File > Open > Image**, to display the Open Dialog Box.

2 In the Open Dialog Box, double-click on a .tif file from the scanner, or select a file and click **Open**.

You can also open an image file by dragging the file to the Feature Extraction desktop icon.

An image of the scanned microarray contained in the file appears in the Workspace.
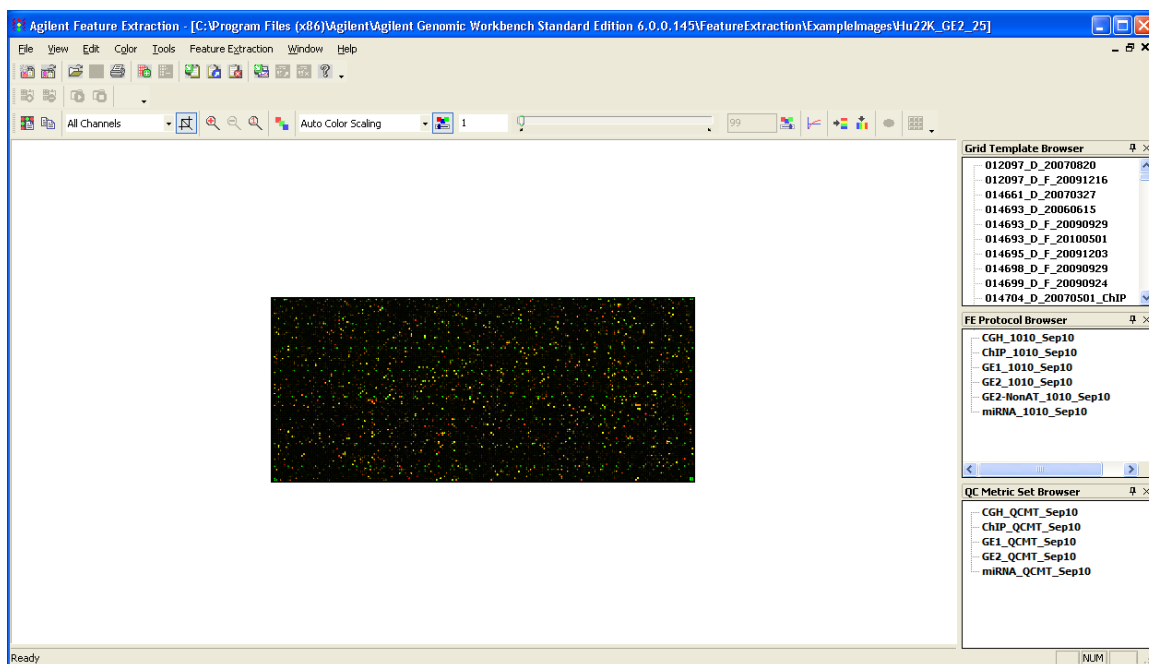
Figure 76   Uncropped TIFF image for Agilent microarray

You can also open an image file double-clicking the image file in a project extraction set. For information on how to do this, see Chapter 2, "Extracting Microarrays Automatically".

To crop an image

Crop an image using the cursor

You do not have to crop an Agilent image before you load it into a project and extract. You may want to crop images to view a subset of features or to use for illustration purposes.

When you first open an image, the Cropping Mode button is turned ON because this is the default setting. (See "To change default image viewing settings" on page 287.)

1 If not already on, click the **Cropping Mode On/Off** button.

2 Position the crop cursor at the top left corner of the image that you want to crop, and hold the left mouse button down.

3 Drag the crop cursor to the opposite corner.

4 Release the left mouse button to display the cropped image.

Cropping mode ON

Figure 77   Image top menu and toolbar in Feature Extraction Tool

The Status Bar is located at bottom of the Feature Extraction window and contains the pixel information about the viewed image. If the viewed image is the preview image, the pixels are from the preview. If the viewed image is the high-resolution image, the pixels are the high-resolution pixels.

If you are viewing high-resolution images, you must crop them to one-tenth or less (i.e. at least 200%) of their original size in order to see the features at high resolution. Any larger cropped image is a preview image.

The color of the crop cursor helps you recognize the proper size for the high fidelity image. If the cursor is black, the cropped image is the high fidelity image. If the cursor is red, the cropped image is still a preview image.

You can cancel the cropping operation by using the (Esc) key, or click the **Cropping Mode On/Off** button to turn cropping off.

Crop an image using corner locations (manual crop)

This option lets you crop an image with greater precision than you can attain with the cursor option.

NOTE For manual cropping to work, the image after cropping must be a minimum of 30 x 30.

1 Click **Edit > Manual Crop**.
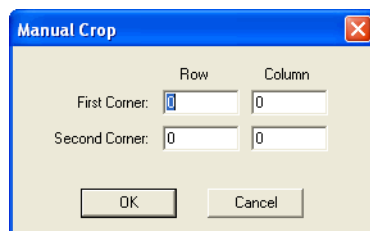
Figure 78 Manual Crop dialog box

2 Enter the locations of the corners (diagonal to one another) that you intend to have as the corners of the cropped image.

3 Click **OK**.

To save a cropped image to a new file

If the image file is from an Agilent microarray with a barcode:

1 Click **File > Save Modified Image** to save the cropped image to a new file.
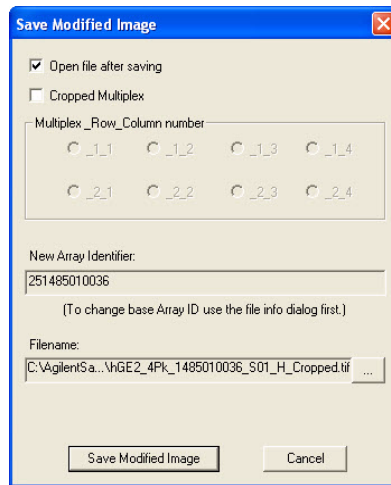
Figure 79   Save Modified Image dialog box for Agilent microarray

2 To close the file after saving, clear the **Open file after saving** check box.

3 To specify which pack in the multiplex (formerly known as multipack) microarray was cropped, select a Multiplex_Row_Column number.

This lets you save the cropped single pack with its multiplex coordinates.

If no multiplex was cropped, clear the Cropped Multiplex check box.

4 To change the New Array Identifier, see "To add or change the New Array Identifier" on page 263.

For Agilent microarrays with a barcode, the New Array Identifier is automatically generated from the barcode and displayed, along with a suggested file name.

The New Array Identifier can be changed. In fact, if for any reason the barcode does not generate a New Array Identifier, you must enter one if you intend to send the MAGE-ML results file to Resolver.

5 To change the path for the cropped image:

- • Click the ellipsis (**...**) button.
- • Select the new path.
- • Click **OK**

6 Click **Save Modified Image**.

If the image file is from a non-Agilent microarray:

- • Click **File > Save Modified Image** to save the cropped image to a new file.

For microarrays without an identifier, the New Array Identifier field is blank. You can add an identifier. See "To add or change the New Array Identifier" on page 263.
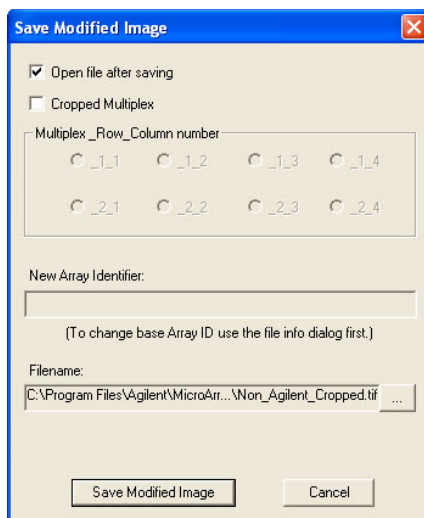
Figure 80   Save Modified Image dialog box for non-Agilent microarray

To add or change the New Array Identifier

When you attempt to save a modified image, the Save Modified Image dialog box appears with a field for New Array Identifier. Agilent barcodes automatically generate this number. For non-Agilent images, this field is blank.

If the New Array Identifier is not generated, for whatever reason, the software will not transfer MAGE-ML or GEML results files to a third-party program such as Resolver.

You can change or add this identifier in the Scan Image Properties dialog box.

1 Click **View > Image File Info** to bring up the Scan Image Properties dialog box.

2 Click the cell next to the **Identifier** field, and enter the new number.

3 Click **Apply**, and click **Close**.

A message appears that says you must click **File > Save As** before the change is accepted.

4 Click **OK**, and click **File > Save As**.

5 Enter a new name or select the old name of the .tif file, and click **Save**.

If you were trying to save a modified image, try again. The New Array Identifier you entered appears in the field.

Loading Feature Extraction visual results

You can select to store visual results in the same local folder as the other types of result files or to have it be sent via FTP to a remote directory. In either case the visual results file is saved with the same name as the results file name with an extension of .shp.

By default, the software shows the results for outlier features only when you load the visual result file or shape file (.shp) for the image. To view all results, you must clear the **View Outliers Only** check box. It is recommended, the you first crop out a region of the image before clearing the outlier check box. For instructions, see "To change the magnification (zooming)" on page 279 and "To change the extraction results display" on page 282.

1 Open the image for which you want to see the visual result.
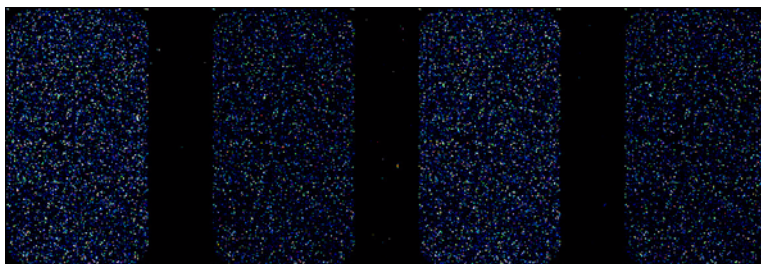
Figure 81   Image of Agilent 1-color, 4x44K microarray

2 Click **Feature Extraction > Load Visual Result** to load previously saved visual results for the currently displayed image file.

The software opens the folder containing the image file whose visual results you want to view. If you chose to save the output files for the project to a results folder separate from the image file folder, you must move to that folder.

3 Select the shape file, and click **Open**.

Or, you can also open the visual result file that is associated with the extraction set from the Project Explorer.

1 From Project Explorer, right-click the image.

2 Click **Load Visual Results**.

This opens the image and loads the shape file associated with that extraction set.

To view visual results for low-resolution images:

1 After .shp file is loaded to the image, zoom in until you see enough of the spots to help you evaluate the results.

2 Pass cursor to feature of interest and hold for a few seconds.

A description of the feature appears.

To find a specific feature, use Ctrl-F. Enter the Feature number of the specific feature you want to find.

Figure 82   Visual results for low-resolution microarray image

To view visual results for high-resolution images (2- by 3-micron or 20-bit):

After you first load the image, you, in fact, are viewing a preview image. You can only view the original high-fidelity image if you crop the size of the image to one-tenth or less of the original size.
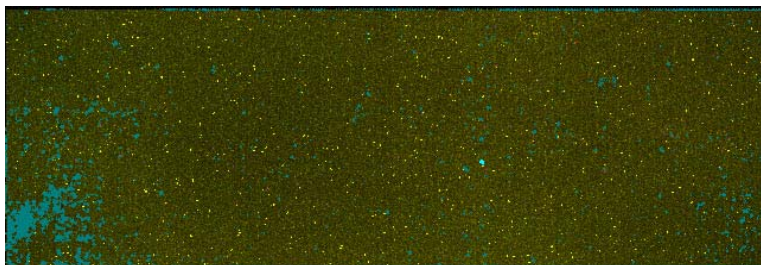
Figure 83   Preview display of high-resolution image with outliers in blue

1 After .shp file is loaded to the image, zoom in until you see enough of the spots to help you identify the region of interest.

2 Click the left mouse button at the point where you want to start forming a rectangle for cropping.

3 With the crop cursor, draw a rectangle equal to or less than one-tenth the size of the image.

Note that the crop cursor is black if the size is within one-tenth (200% to 1000%) of the original size, but it turns red if you are drawing outside the high fidelity range.

4 Then release the mouse button.

Note the status bar at the bottom of the window. It should now read "**High Fidelity**".

High fidelity mode is when you zoom in the image by at least 200%.

5 Pass cursor to feature of interest and hold for a few seconds.

A description of the feature appears.
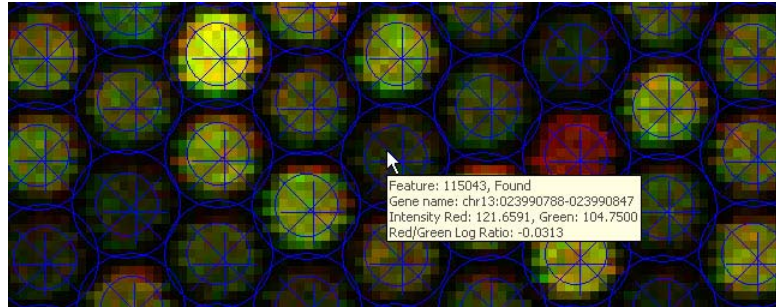
Figure 84   Visual results of high-resolution microarray image in
High Fidelity mode, zoomed to 500%

View pixels used

This view option for low-resolution images and for
high-resolution images that are in "High Fidelity" mode (i.e.
when the image is zoomed in to one-tenth of its original
size).

The **View Pixels Used** option displays the pixels used to
quantify the feature signal within the cookie area. Pixels that
are determined to be outliers are shown in black.

1 If the image is a high resolution image, complete steps
1-4 from "To view visual results for high-resolution
images (2- by 3-micron or 20-bit):" on page 266.

2 Under **View > Extraction Results**, select **View Pixels
Used**.

To view visual results for low-resolution images:
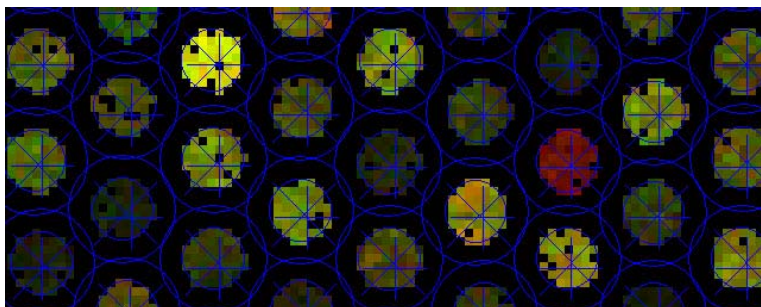
Figure 85   View pixels used for high-resolution microarray image in
High Fidelity mode, zoomed to 500%

To save an image (original or cropped) for import to another program

1 Click **File > Save As** to produce high resolution images.

The Save As dialog box lets you save .tif files or .jpg files of the current image or cropped image.

2 Click the down arrow to the right of the **Save as type:** list.

3 Select either **Tif Files, BMP files Png Files,** or **Jpeg Files**.

4 Enter the name of the image.

5 Click **Save**.
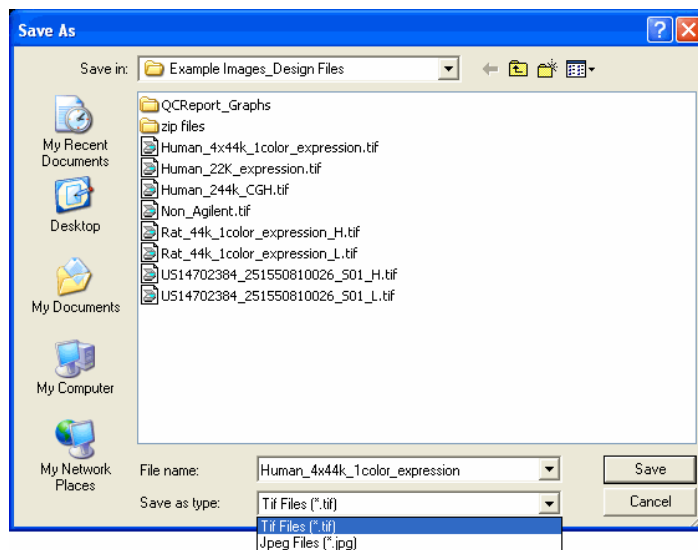
Figure 86   Save As dialog box

To display file information

You can use the File Info dialog box to view valuable information, such as scanner warning messages, scanner version, PMT voltage, dark offset information, the scan resolution and glass thickness

• Click **View > Image File Info** to display a tabbed dialog box that displays all available information about an image file.
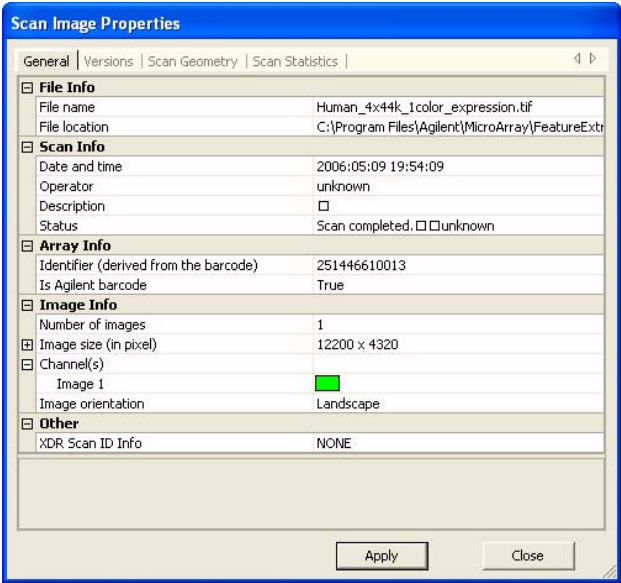
Figure 87   Scan Image Properties dialog box

The only field that you can change is the Identifier field. See

Changing image display settings

Changing the image display does not affect the Feature Extraction results in any way.

When you open an image you can manipulate displayed images from the original scan file, the cropped scan file or the Feature Extraction results in the following ways:

- Change image orientation (landscape/portrait)
- Change the number of channels displayed
- Change the display color
- Change between log and linear color scale
- Change the intensity scale (color display range)
- Change the magnification (zooming)
- Change the fit of the window to the image
- Change the extraction results display

This section also shows you how to change settings for histograms and line graphs, the default image analysis parameters and printing.

To change image orientation (landscape/portrait)

Due to the size of 2um or 3um scan images, the flip and rotate operation can take several minutes.

When you first load a microarray image into the Feature Extraction Tool, the image appears in the orientation produced by the scan, either landscape or portrait. If the gene list that you want to use to position a grid and find spots has a different orientation than the image, you must change the image orientation to match that of the gene list.

Flip and rotate of 2um, 3um, and 20-bit scans is supported in v10.7 and above.

1 Click **Tools > Flip Upper Left to Lower Right (Landscape/Portrait)** from the top menu.

If the original orientation was landscape, the software takes the upper left corner and moves it to the lower right corner to create a portrait orientation. The same is true if you go from a portrait to landscape orientation.

2 Click **File > Save modified image**.

If you do not save the image in its new orientation, you could run into problems with Feature Extraction later.

To change the channels that display

The default setting for displaying the channels is to display one window with the two color channels combined.

To change the channels for a single window:

1 On the image viewing toolbar click the down arrow for the **Channel Display** menu.

The default selection is All Channels.

2 Select **Red** Channel or **Green Channel**.

The window display changes to the channel selected.

You can also change the number and color of the channels displayed when you set default image settings.

To display separate channel images:

Displaying the images of separate color channels side by side can be useful for comparing background anomalies on the microarray.

1 Click **Window > New "Filename" View Window**.

"Filename" represents the path and name of the image that you loaded. The Select Image dialog box appears.
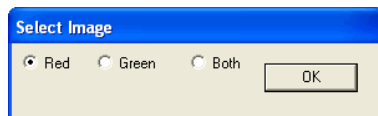
2 Click **Red** or **Green**.

3 Click **OK**.

If you repeat steps 1-3, all three windows—the red, the green and the combined color—display at the same time.

To change the display color

"Color Mode" choices are False Color, Gray Scale, Reverse Gray Scale and False Color w/White BG.

• From the image viewing top menu, click **Color > "color mode"**.
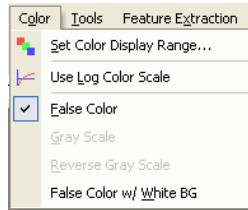
Figure 88   Color menu

The color type that you select depends on the channel image display. For combined channels, there are two choices of color, and for single channels there are three choices.

Combined channel display

The default setting for the color on the TIFF image is called False Color. You also have the option of selecting False Color with a white background.

• Click **Color > False Color w/ White BG**.

To return to False Color, click **Color > False Color**.

False Color   This selection displays 1-color images using a color palette that progresses from black intensity (zero) to blue to cyan to green to yellow to red to magenta intensity (65535). For 2-color images, the red channel is displayed as shades of red and the green channel as shades of green that are combined to show shades of yellow.

False Color w/ White BG   This selection maps intensities the same as the False Color palette, except that a pixel with zero values in both red and green is turned white instead of black. This feature is useful when printing because it saves ink. Generally, the minimum data range must be manually adjusted to produce the best possible image.

Single channel display

The default setting for the color on the single-channel display is also False Color.

- Click either **Color > Gray Scale** or **Color > Reverse Gray Scale**.

Gray Scale   Presents the spot intensity in white and the background in black.

Reverse Gray   Presents the spot intensity in black and the background in white.

To return to False Color, click **Color > False Color**.

Examples of images with different color selections

The following images are intentionally not aligned.
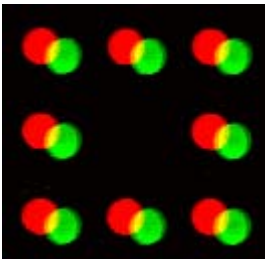
Figure 89   False Color for 2-color image
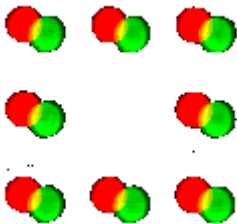
Figure 90   False Color with white BG for 2-color image
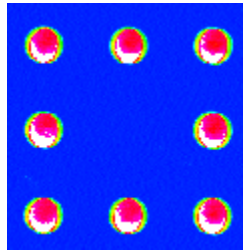
Figure 91   False Color for 1-color image
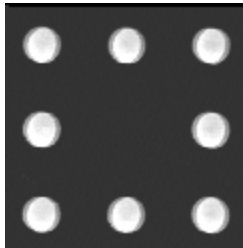
Figure 92   Gray Scale
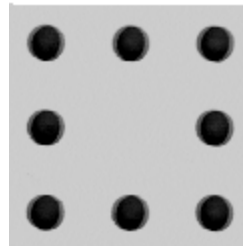
Figure 93   Reverse Gray Scale

To change between log and linear color scale

- Click **Color > Use Log Color Scale** to toggle the color scale from linear to $\log_{10}$ (intensity).

  To return to the linear scale, click **Color > Use Log Color Scale** again to remove the check mark.

  With the log scale you can see low intensity portions of the microarray at the expense of detail at high intensities.

  You can also use the Log/Linear button on the toolbar to toggle the color scale.

To change the intensity scale (color display range – CDR)

Changing the color display range affects how the image intensity is scaled. Modifying the color display range can make it easier to visualize your microarray image but does not affect the raw intensity.

You can change the color display range in one of two ways:

- Using the color scaling toolbar.
- Using the Set Color Display Range dialog box.

Use the color scaling toolbar

The easiest way to determine the best range is to use the Auto Scale function on the toolbar.

CDR Low Mode Icon   CDR High Mode Icon
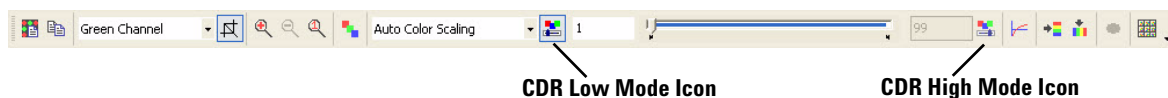
Figure 94   Color scaling segment of the image viewing toolbar

1 Click the down arrow next to the color scaling menu, and click **Auto Color Scaling** if necessary.

2 Click the **CDR Low Mode** icon, and move the lower bar cursor to the minimum percentage for the display range.

3 Click the **CDR High Mode** icon, and move the higher bar cursor to the maximum percentage for the display range.

Notice the change in color of the image display as you increase or decrease the blue bar size.

4 To fine tune the color display ranges manually, click either the **Red Channel** or the **Green Channel** from the color scaling menu.

5 Click the **CDR Low Mode** icon, and move the lower bar cursor to the minimum pixel number for the display range.

6 Click the **CDR High Mode** icon, and move the higher bar cursor to the maximum pixel number for the display range.

Use the Set Image Color Display Ranges dialog box

Alternatively, you can set the color display range through the Set Image Color Display Ranges dialog box.

1 Click **Color > Set Color Display Range**, or click **Set Color Range** on the toolbar.
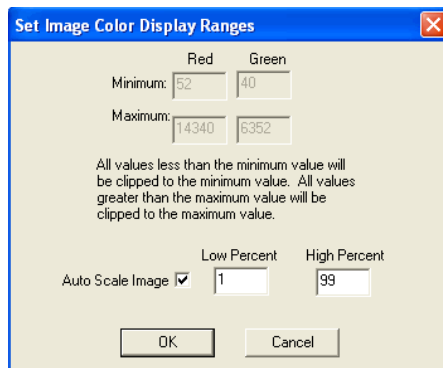
Figure 95 Set Image Color Display Ranges dialog box

2 Mark the **Auto Scale Image** check box to set a low and a high percentage cutoff value.

Setting 0 for a low value and 100 for a high value will compress the entire range of possible values into the 8-bit displayable range. If this produces a black image, try 1 and 99 (the default settings). Generally, this gives you an acceptable data range, even for very poor images.

3 To fine tune the color display ranges manually, clear the **Auto Scale Image** check box.

4 Change the **Minimum** and **Maximum** Red and Green values.

The data range determines how the 16-bit image data is mapped to the 8-bit data that is displayed on a computer screen. Generally, scanned images do not cover the entire range of possible values: 0 to 65535. Therefore, if you try to display the entire range, all you can see is black.

To change the magnification (zooming)

To set the default % zoom settings for the loaded image and the grid mode images, see "To change default image viewing settings" on page 287 of this guide.

Magnification can only be set to one of the % zoom levels shown in Figure 96. Numbers greater than 100% map one scan pixel to multiple pixels on the screen. For example, 600% maps one scan pixel to a 6 x 6 square of identical pixels. A zoom value of 100% maps one scan pixel to one pixel. Values less than 100% display the mean value for several scan pixels to one pixel. For example, 50% averages four scan pixels, a 2 x 2 matrix, and displays the average as one pixel.

You can control zooming in one of three ways:

- Dragging the cursor to make a rectangle
- Using buttons on the tool bar and on the mouse
- Using the View menu

Drag the cursor to make a rectangle

You can only zoom the image using this method if cropping is turned off.

1 Clear the **Cropping Mode On/Off** button to turn cropping off.

Cropping mode OFF
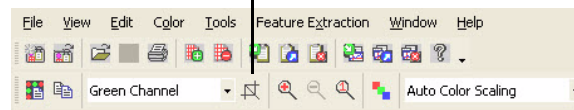
2 Position the cursor at the top left corner of the image section that you want to zoom, and hold the left mouse button down.

3 Drag the cursor to the opposite corner of the section you want to zoom.

4 Release the left mouse button to display the selected area.

Use the Tool Bar and Mouse Buttons

Three buttons on the tool bar and the right and left mouse buttons control zooming. You can use these buttons with Crop Mode On or Off.

Use the Zoom Menu

You can use the Zoom menu with Crop Mode On or Off.

• Click **View > Zoom** to jump to any zoom level directly.

For low-resolution images, if the image is uncropped, the zoom levels range from 10% to 1000%.

For high-resolution images, if the viewed image is uncropped, the zoom levels range from 10% to 100%. To activate zoom levels from 200% to 1000% (High Fidelity mode), you must crop the image.

Note that the crop cursor is black if the size is within one-tenth (200% to 1000%) of the original image size, but it turns red if you are drawing outside the High Fidelity range.
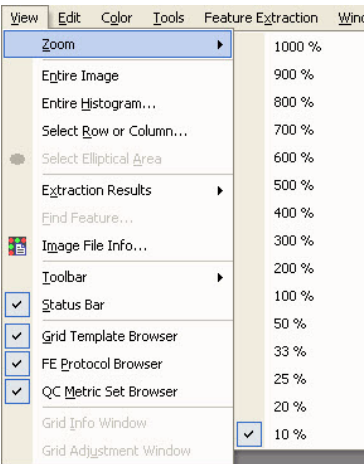
Figure 96   Zoom menu for low-resolution image

To change the window size to fit the image

If you have to scroll to see the entire image, use this option to increase the size of the window to fit the image. If the window is too big for the image and contains white space around the perimeter of the image, use this option to decrease the size of the window to fit the image.

• Click **View > Entire Image** to resize the window.

To change the extraction results display

Use this option to control the portions of the feature extraction results that are displayed.

• Click **View > Extraction Results**.
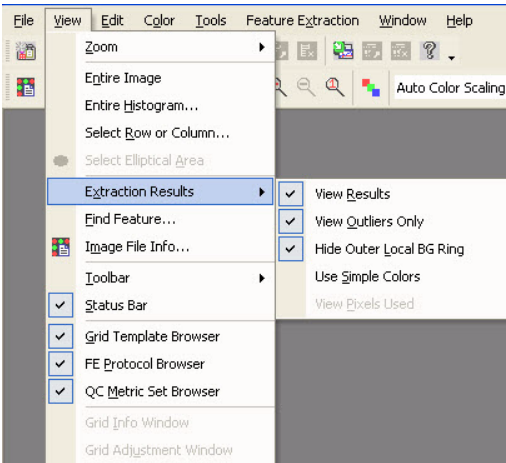
Figure 97   Extraction Results — Default views selected

View Results   Results shown depends on whether **View Outlier Only** is selected or not.

Shows all visual results when this option is selected and **View Outliers Only** is not selected.

Shows outlier results only when both this option and **View Outlier Only** are selected.

Hide all visual results when this option is not selected, even if **View Outliers Only** is selected.

View Outliers Only   Shows outlier features only when both this option and **View Outliers Only** are selected.

Hide Outer Local BG Ring   Shows or hides the local background ring. See "To select a spot statistics method to define features" on page 208 of this guide for a definition of the local background.

Use Simple Colors   Toggles the color display of outlier features and background.

View Pixels Used   Shows or hide pixels within the cookie that are used to quantify the feature mean or median intensity.

For more details, see "View pixels used" on page 267.

To change and save histograms and line graphs

For high-resolution images, histograms and line graphs use the original data, not the data from a low-resolution preview image.

Set up a histogram graph

Histograms can be very useful to initially visualize your results. They can show you if you have an anomalous distribution of intensities across the entire slide or across rectangular or elliptical subregions of the image.

Entire viewing area

1 Click **View > Entire Histogram** from the main menu bar.

   If the selected file has more than one color, a Channel Selection dialog box appears.

2 Select the desired channel, or select both.

Rectangular subregion

1 Make sure that cropping is OFF.

2 Click the **right** mouse button to position your cursor in one corner of the region to capture.

3 Hold down the right button and drag the pointer to the opposite corner, then release.

   If the selected file has more than one color, a Channel Selection dialog box appears.

4 Select the desired channel, or select both.

Elliptical subregion

1 Click **Tools > Preferences > Graph Viewing.**

2 Click the cell next to **Select Elliptical Area for Histogram**, and click the **Up** arrow.

3 Select **True**, and click **OK**.

False is the default setting and results in a rectangular subregion.
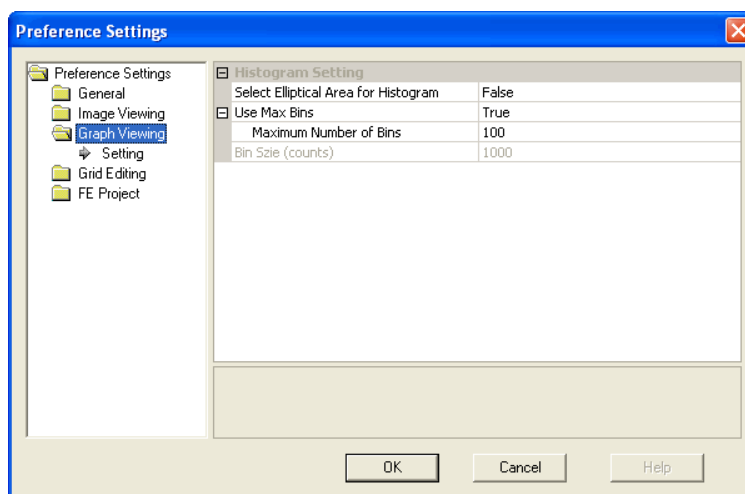
Figure 98   Histogram Preference Settings dialog box

4 Make sure that cropping is OFF.

5 Click **H** .

Or, click **View > Select Elliptical Area**.

6 Click the right mouse button to position your cursor in one corner of the region to capture.

7 Hold down the right button and drag the pointer to the opposite corner, then release.

If the selected file has more than one color, a Channel Selection dialog box appears.

8 Select the desired channel, or select both.
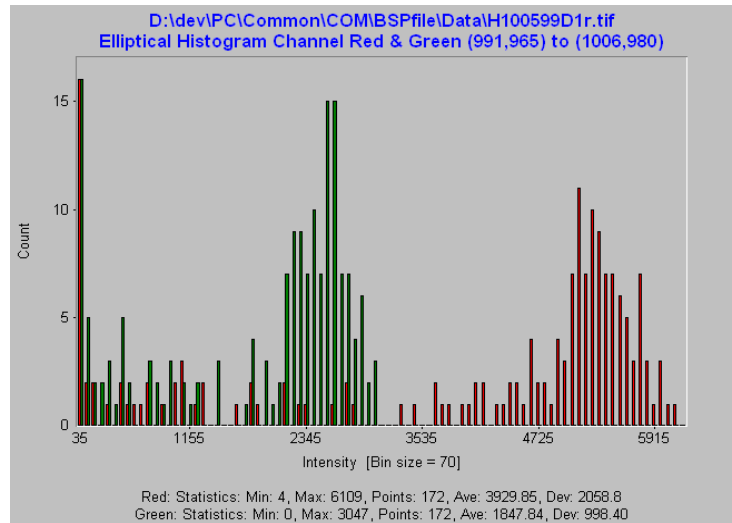
Change the bin size for histograms

1 To adjust the bin size after creating the initial graph, click **Histogram > Change Bins**.

2 Change the bin size or the maximum number of bins.

3 Click **OK**.

The maximum bin size is 65535; the minimum is 1.

The maximum number of bins is 32768; the minimum is 2.

Change the default bin size for histograms

1 Click **Tools > Preferences**.

2 Click the **Graph Viewing** folder.

3 Change the bin size or the maximum number of bins.

4 Click **OK**.

Set up a line graph

Line graphs show a vertical or horizontal slice through the image. They can be useful for spotting extreme differences between the red and green signal intensities across the microarray. Line graphs are also useful for displaying average intensity over all rows and columns, minimizing the effect of noise.

To create a line graph more quickly, double-click a selected row within the image with the left mouse button, or double-click a selected column with the right mouse button.

1 Click **View > Select Row or Column**, or click or .

The Select Row/Col dialog box appears.
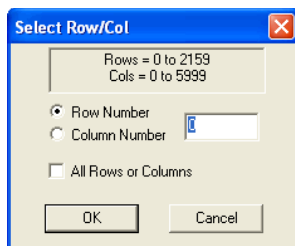
Figure 99 Select Row/Col dialog box

2 Click **Row Number** for showing signals in a row, or click **Column Number** for showing signals in a column.

3 Enter the number of the row or column.

4 Mark the **All Rows or Columns** check box to average the rows or columns in the view depending on the Row Number or Column Number selection.

5 Click **OK**.
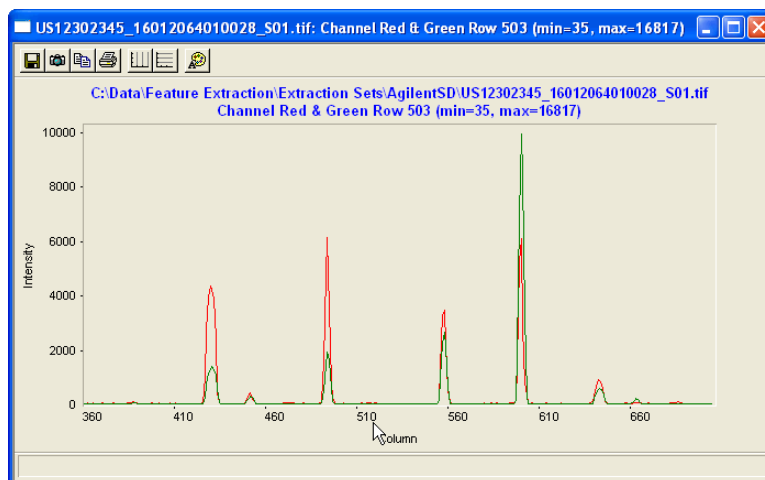
Figure 100 Line graph example

Use the Graph Toolbar

You save graphs produced from the image files to third party programs, such as word processors and spreadsheets, with the buttons in the Graph Toolbar. You can also print and modify the graph display with this toolbar.

• Click the selected button in the Graph Toolbar at the top of the histogram or line graph.

To change default image viewing settings

You can also change the default settings that appear when you open a new file. To activate the settings you must exit the image file and either reopen the same file or open a new one.

1   Click **Tools > Preferences**.

2   Open the **Image Viewing** folder, if necessary.

3   Change the default selections if you need to.

4   Click **OK**.

5   Close the image.

6   Reopen a file.

   The new default settings now take effect.
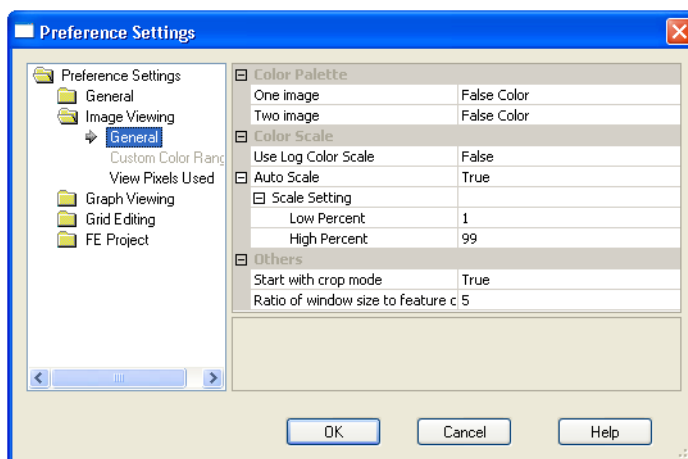
Figure 101   Image View Default Options dialog box

Color Palette   You can set the color for the images found on a microarray.

Use log color scale   If set to True, the software uses the log10 intensity scale for the color. If False, it uses a linear scale.

AutoScale   If set to True, you can change the Low Percent and the High Percent for autoscaling the color display range.

Start with crop mode   Select True if you want to be able to crop the image with mouse movement right after you load the image. Select False if you want the mouse to activate the zoom function.

Ratio of window size to feature cross for found feature   When you attempt to find a feature in the visual result image, you can change the ratio of the resulting window size for the image to the size of the feature cross for this found feature.

Change other default settings in the Preferences dialog box

See the tasks in the relevant chapters for changing the default settings for the Grid Mode (Chapter 2), for Graphs (histogram bin settings in this chapter) and for Projects (Chapter 1).

To change the print settings

The Feature Extraction application supports printing to both color and black and white printers.

Change page settings

• Click **File > Page Setup** to set the following printer paper parameters:

  • Paper size
  • Paper source
  • Paper orientation
  • Margins
  • Printer

Change print settings

1 Click **File > Print Setup** or **File > Print** to bring up the standard Windows Print Setup dialog box.

  Within this dialog you can change the following parameters:

  • Paper size
  • Paper source
  • Paper orientation
  • Margins
  • Printer

2 Click **OK** on the Print Setup dialog box to save changes to the printer setup without printing, or

Click **Print** to save changes to the printer setup and to print.

Preview a file before printing

• Click **File > Print Preview**.

Printing single color images

If you are printing a single color image that is displayed using the false color palette to a black and white printer, the Image Analysis Tool of Feature Extraction automatically converts the image to gray scale. This is necessary because the gray scale intensities that a black and white printer automatically generates for a false color image are incorrect.

Examples   The following images are intentionally not aligned.
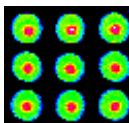
Figure 102   False Color image
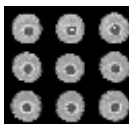
Figure 103   Incorrect image generated by blank and white printer
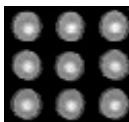
Figure 104   Correct gray scale generated by the Image Analysis Tool

Printing 2-color images

Printing 2-color images on a black and white printer does not require the Image Analysis Tool to convert the image to gray scale, but you cannot distinguish between the colors. See the following example.

These images are intentionally not aligned.

Figure 105   2-color image

Figure 106   2-color image printed on a black and white printer

Images always print at maximum resolution. The zoom level does not affect printing. This resolution setting makes printing the image slow but also generates the highest quality image possible.

Hot Key actions for image displays

Table 11   Hot key actions for image displays

If you want to do this:	Press this "hot key":
Turn Crop Mode On/Off	Alt-C
Call up the manual crop dialog box	Ctrl-M
Call up the File Info dialog box	Ctrl-I
Change image orientation (landscape/portrait)	Shift-U
Zoom in	Ctrl-double-click left mouse

Table 11   Hot key actions for image displays

If you want to do this:	Press this "hot key":
Find feature when viewing extraction results	Ctrl-F
Open new window Does not work for high resolution scans (2um, 3um, or 20-bit)	Ctrl-N
Open file	Ctrl-O
Print TIFF image	Ctrl-P
Save the image as a different file	Ctrl-S

Changing Feature Extraction window displays

When you start the Feature Extraction software, you see the default window layout that Agilent configured before shipment. This window layout always appears unless you move windows and panes and change the default settings in the Preferences dialog box.
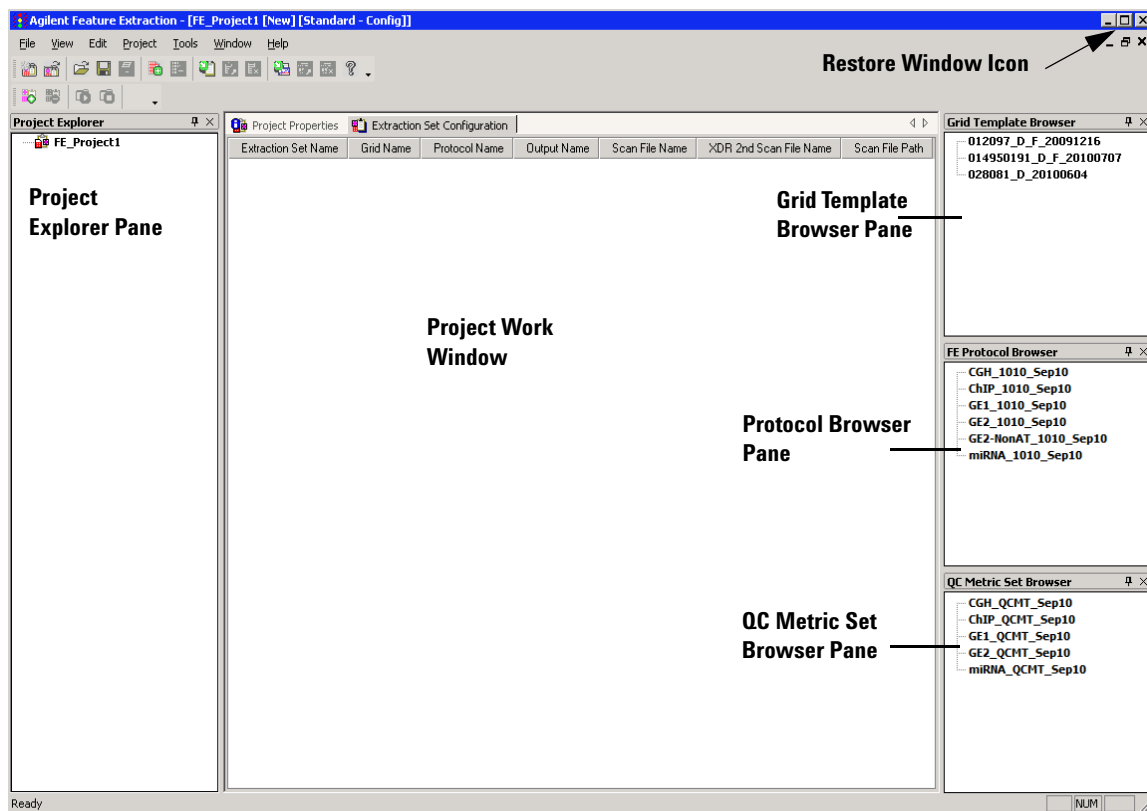
Figure 107   FE Standard Project Configuration window – default

Below is another layout of the FE Standard Project Configuration window. This section shows you how to change from one layout to a new layout and to save the new layout so that it appears when the software starts.
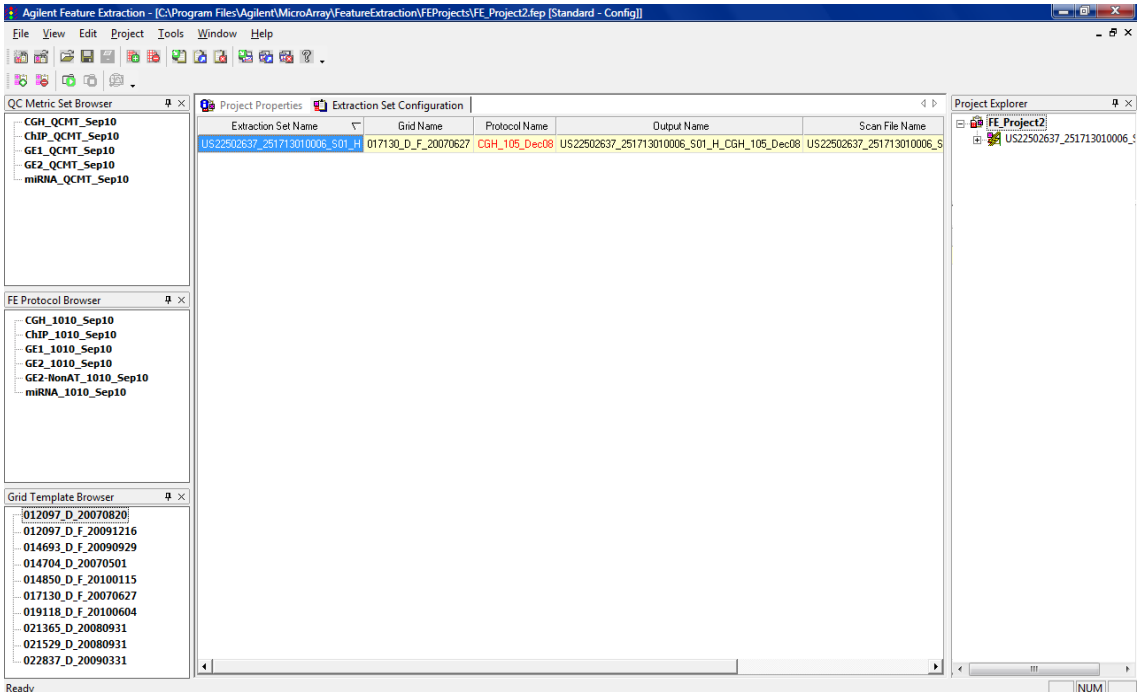
Figure 108   FE Standard Project Configuration window – new layout

To move windows and panes

Windows can be separated from window frames and blended into frames ("maximized").

• Click the **Restore Window** icon for the Project Work Window. See Figure 107.

The Project Window becomes separated from the main window frame, exposing the Workspace.

Panes can be unattached from one edge of a window frame and docked to another edge of the frame.

1 Click on the title of the Grid Template Browser Pane and drag it from the right edge of the window frame.

2 Move the Browser across the Workspace until the **cursor** hits the left edge of the frame, then release the mouse button.

The Grid Template Browser is now "docked" to the left edge of the frame. You can dock a pane to any edge of the frame in this way.

3 Repeat step 1 for the FE Protocol Browser Pane.

4 Move the Browser across the Workspace and place the **cursor** on the title of the Grid Template Browser.

Now both panes are overlapping one another with tabs at the bottom of the panes. To separate the two panes once more, click on a tab and move it to a new position.

If you had moved the FE Protocol Browser Pane to the left edge of the frame, the two Browsers would be next to each other.

5 Click on the title of the Project Explorer Pane and drag it across the Project window until the cursor hits the right edge of the window frame, then release the mouse button.

Your screen should now look like Figure 108.

Two other actions can be taken with panes:

• Close panes. Click on the X in the upper right corner.

• Autohide panes when docked. Click on the pin icon in the upper right corner of the pane.

The pane now appears as a bar button on the edge to which it was docked.

When you click on the resulting bar button, the pane floats from the edge so that you can view its contents. Note that the pin position is now horizontal instead of vertical. If you click on the pin now, the pin becomes vertical and the pane redocks itself to the edge of the frame.

To select the layout to use when software starts

You set the default layout for the Feature Extraction display in the Preferences dialog box.

1 Click **Tools > Preferences** from the top menu.

2 Change the following settings to produce the layout you want upon software startup.

If you want to return the windows and panes to their original positions at any time, set "Frame Windows Using last saved Position" and "Docking Pane Using last saved Layout" to FALSE, close Feature Extraction and reopen the software.
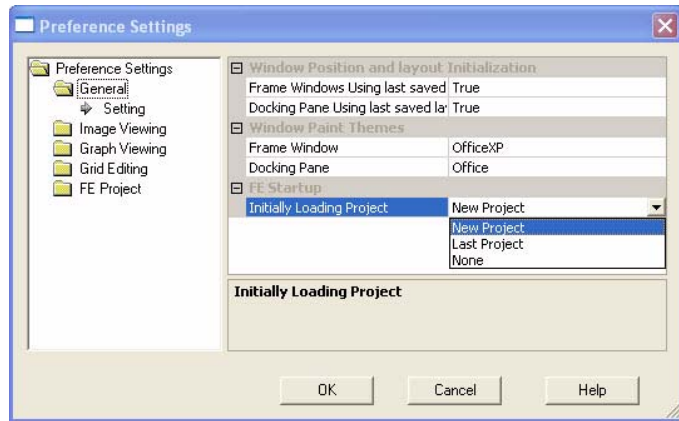
Figure 109   Preference Settings dialog box

Frame Windows Using last saved Position	If **True**, the window positions you have changed in the last session appear upon startup. If **False**, the factory default window position appears. To have Figure 108 appear upon startup, keep the setting as True.
Docking Pane Using last saved Layout	If **True**, the docking positions from the last session appear upon startup. If **False**, the factory default docking positions appear. To have Figure 108 appear upon startup, keep the setting as True.
Initially Loading Project	If **New Project**, FE Project1 always appears upon startup. If **Last Project**, the project on the screen before exiting appears at startup. If **None**, the Workspace is blank upon startup. Keep New Project to reproduce Figure 108.

5 Changing Image Displays

To select the layout to use when software starts

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In This Book

The *User Guide* presents instructions for using the the Agilent Feature Extraction software 10.10.

This guide provides instructions for:

- Setting up and running Feature Extraction on batches of image files.

- Creating grid files to assign to image files for automatic extraction.

- Changing the parameter values for each step in a protocol that can be assigned to an image file for automatic extraction.

- Working with image displays.

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