Feature Extraction for CytoGenomics 2.9

Scanner settings and feature extraction recommendations for Innopsys InnoScan 710 and 900 microarray scanners

For Research Use Only. Not for use in diagnostic procedures.

CytoGenomics 2.9 can process tiff image data from Agilent microarrays scanned on Innopsys InnoScan 710 and 900 scanners. To analyze these images in Agilent CytoGenomics, use the scanner settings and feature extraction recommendations provided here.

Agilent Technologies

Scanning an Agilent CGH array on an InnoScan 710 or 900 scanner

If you will be scanning Agilent microarrays on an InnoScan 710 or 900 scanner, follow the recommendations provided below when scanning your arrays.

Scan for two colors simultaneously

In the Mapix software, on the General tab of the Scan Configuration window, make the following selections to scan for two colors.

- Mark **Automatic scrolling**
- Mark **Barcode reading**
- Select **3.0** for Pixel size
  - Agilent G3 Arrays (1x1M, 2x400K, 4x180K, and 8x60K) - 3 µm resolution
- Select **10** for Speed
- Select **Normal** for Scan Mode
- Select **Simultaneous** for Acquisition Mode
- In Manual mode, select Low Laser Power and 100% Detection gain for both 635 and 532
- In Auto mode, select **% of saturated pixel** and set the value to 0.3.

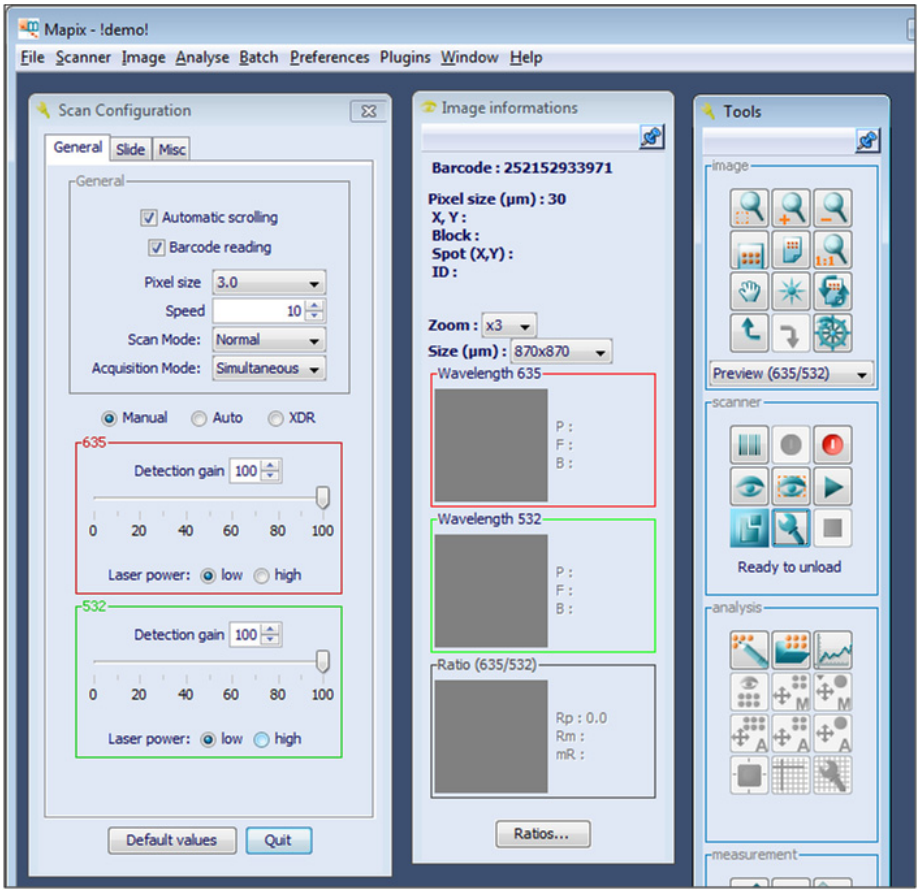


Figure 1 Mapix – Scan Configuration window – General tab

Do not crop the scan area

Follow the recommendations below when defining the scan area in the Mapix software.

- In the Preview window, define a scan area that captures the entire feature area.
- On the Slide tab of the Scan Configuration window, select **Labeled slide** and define a 10 mm label height.

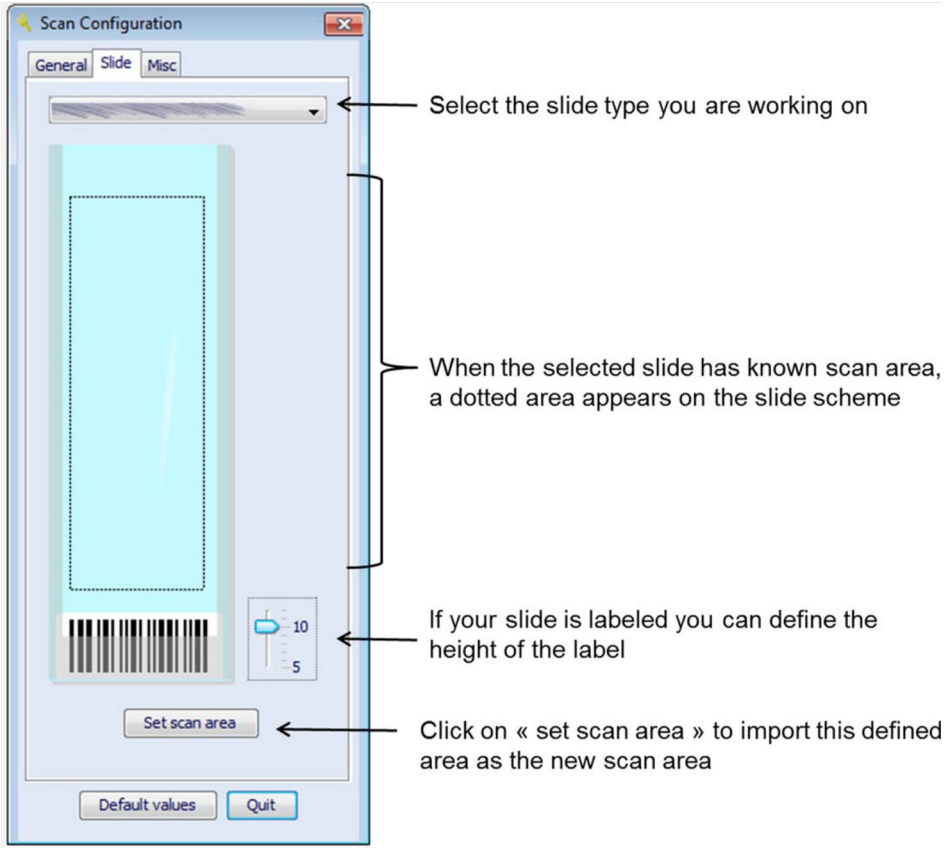


Figure 2 Mapix – Scan Configuration window – Slide tab

- When starting the scan, in the Save window, ensure that **Separated TIFF files** is not marked. Agilent Feature Extraction can only convert single TIFF scans from non-Agilent scanners.
- Mark the following check boxes in the Save window.
  - Prefix File Name
  - Barcode
  - Date & Time

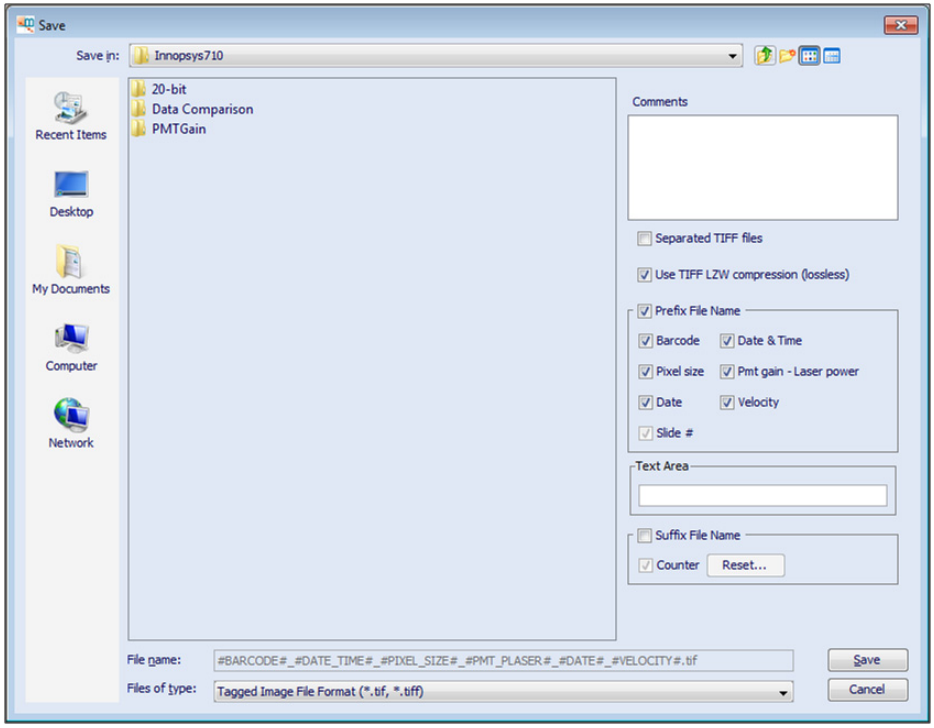


Figure 3 Mapix – Save window

Running a workflow with the scanner image in CytoGenomics

Manually run a workflow in CytoGenomics

See the CytoGenomics help system for instructions on manually running a workflow. An overview of the basic steps is provided below.

1. In CytoGenomics 2.9, on the Analysis Workflow screen, click **Select Samples** and select the tiff image (*.tif) generated on the InnoScan scanner. Click **Next** to advance to the Describe Samples step.
2. Assign the sample attributes as needed. Click **Next** to advance to the Run Analysis step.
3. Add a description to the workflow job (if desired), and click **Run Analysis** to start the workflow. The program directs you to the Sample Review screen where the submitted workflows are listed.

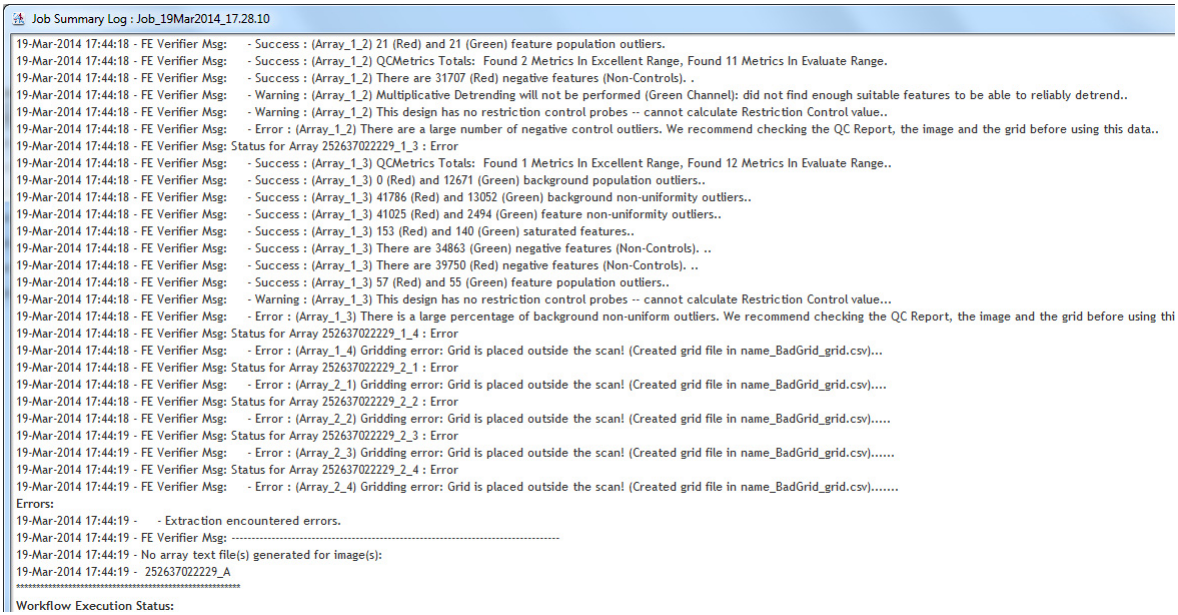
Check the status of the workflow

Workflows using images from an InnoScan scanner sometimes fail due to failure to automatically grid the image. When an extraction fails due to auto gridding, all of the submitted workflows for the image(s) will show a value of “Failed” in the Status column of the Sample Review screen in CytoGenomics.

To check if your workflow failed due to automatic gridding errors:

1. On the Sample Review screen, double-click the first failed row.

   A pop-up window opens showing the log of Feature Extraction instructions and the cause of failure.
2. Check the log to confirm if the failure was due to “Gridding error: Grid placed outside of scan!” and verify that auto-gridding was not properly executed. If so, you must manually grid the failed image by running the Feature Extraction application. See “Running Feature Extraction for CytoGenomics” on page 8.


Job Summary Log : Job_19Mar2014_17.28.10

19-Mar-2014 17:44:18 - FE Verifier Msg: - Success : (Array_1_2) 21 (Red) and 21 (Green) feature population outliers.
19-Mar-2014 17:44:18 - FE Verifier Msg: - Success : (Array_1_2) QCMetrics Totals: Found 2 Metrics In Excellent Range, Found 11 Metrics In Evaluate Range.
19-Mar-2014 17:44:18 - FE Verifier Msg: - Success : (Array_1_2) There are 31707 (Red) negative features (Non-Controls). .
19-Mar-2014 17:44:18 - FE Verifier Msg: - Warning : (Array_1_2) Multiplicative Detrending will not be performed (Green Channel): did not find enough suitable features to be able to reliably detrend..
19-Mar-2014 17:44:18 - FE Verifier Msg: - Warning : (Array_1_2) This design has no restriction control probes -- cannot calculate Restriction Control value..
19-Mar-2014 17:44:18 - FE Verifier Msg: - Error : (Array_1_2) There are a large number of negative control outliers. We recommend checking the QC Report, the image and the grid before using this data..
19-Mar-2014 17:44:18 - FE Verifier Msg: Status for Array 252637022229_1_3 : Error
19-Mar-2014 17:44:18 - FE Verifier Msg: - Success : (Array_1_3) QCMetrics Totals: Found 1 Metrics In Excellent Range, Found 12 Metrics In Evaluate Range..
19-Mar-2014 17:44:18 - FE Verifier Msg: - Success : (Array_1_3) 0 (Red) and 12671 (Green) background population outliers..
19-Mar-2014 17:44:18 - FE Verifier Msg: - Success : (Array_1_3) 41786 (Red) and 13052 (Green) background non-uniformity outliers..
19-Mar-2014 17:44:18 - FE Verifier Msg: - Success : (Array_1_3) 41025 (Red) and 2494 (Green) feature non-uniformity outliers..
19-Mar-2014 17:44:18 - FE Verifier Msg: - Success : (Array_1_3) 153 (Red) and 140 (Green) saturated features..
19-Mar-2014 17:44:18 - FE Verifier Msg: - Success : (Array_1_3) There are 34863 (Green) negative features (Non-Controls). ..
19-Mar-2014 17:44:18 - FE Verifier Msg: - Success : (Array_1_3) There are 39750 (Red) negative features (Non-Controls). ..
19-Mar-2014 17:44:18 - FE Verifier Msg: - Success : (Array_1_3) 57 (Red) and 55 (Green) feature population outliers..
19-Mar-2014 17:44:18 - FE Verifier Msg: - Warning : (Array_1_3) This design has no restriction control probes -- cannot calculate Restriction Control value...
19-Mar-2014 17:44:18 - FE Verifier Msg: - Error : (Array_1_3) There is a large percentage of background non-uniform outliers. We recommend checking the QC Report, the image and the grid before using thi
19-Mar-2014 17:44:18 - FE Verifier Msg: Status for Array 252637022229_1_4 : Error
19-Mar-2014 17:44:18 - FE Verifier Msg: - Error : (Array_1_4) Gridding error: Grid is placed outside the scan! (Created grid file in name_BadGrid_grid.csv)...
19-Mar-2014 17:44:18 - FE Verifier Msg: Status for Array 252637022229_2_1 : Error
19-Mar-2014 17:44:18 - FE Verifier Msg: - Error : (Array_2_1) Gridding error: Grid is placed outside the scan! (Created grid file in name_BadGrid_grid.csv)....
19-Mar-2014 17:44:18 - FE Verifier Msg: Status for Array 252637022229_2_2 : Error
19-Mar-2014 17:44:18 - FE Verifier Msg: - Error : (Array_2_2) Gridding error: Grid is placed outside the scan! (Created grid file in name_BadGrid_grid.csv).....
19-Mar-2014 17:44:19 - FE Verifier Msg: Status for Array 252637022229_2_3 : Error
19-Mar-2014 17:44:19 - FE Verifier Msg: - Error : (Array_2_3) Gridding error: Grid is placed outside the scan! (Created grid file in name_BadGrid_grid.csv)......
19-Mar-2014 17:44:19 - FE Verifier Msg: Status for Array 252637022229_2_4 : Error
19-Mar-2014 17:44:19 - FE Verifier Msg: - Error : (Array_2_4) Gridding error: Grid is placed outside the scan! (Created grid file in name_BadGrid_grid.csv).......
Errors:
19-Mar-2014 17:44:19 - - Extraction encountered errors.
19-Mar-2014 17:44:19 - FE Verifier Msg: ----------
19-Mar-2014 17:44:19 - No array text file(s) generated for image(s):
19-Mar-2014 17:44:19 - 252637022229_A
****
Workflow Execution Status:

Figure 4 CytoGenomics – Job Summary Log

Running Feature Extraction for CytoGenomics

The Feature Extraction for CytoGenomics software is installed as part of the Agilent CytoGenomics software package. When you run a workflow from CytoGenomics, Feature Extraction for CytoGenomics runs in the background to automatically extract microarray images. It finds and adjusts microarray grids, rejects outlier pixels, accurately calculates feature intensities and ratios, flags outlier features, and calculates statistical confidences.

Ordinarily, it is not necessary to run the Feature Extraction program separately. However, if your workflows failed due to failure to automatically grid the image, you will need to manually grid the image in Feature Extraction for CytoGenomics before re-running the workflow in CytoGenomics.

Open the Feature Extraction for CytoGenomics program

To start Feature Extraction for CytoGenomics:

- From Windows, click **Start > All Programs > Agilent CytoGenomicsEdition 2.9.x > Agilent Feature Extraction for CytoGenomics**.

  The Feature Extraction for CytoGenomics main window opens.

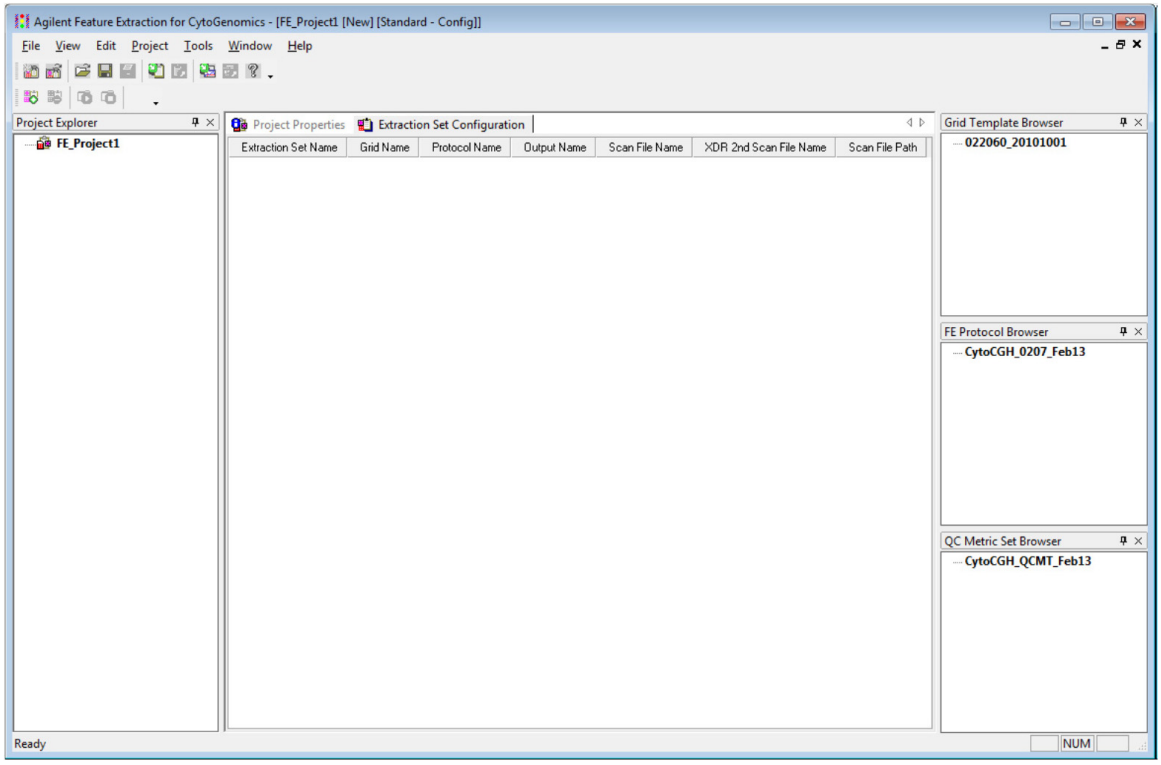


Figure 5 Feature Extraction for CytoGenomics – Main window

Open the image in Feature Extraction for CytoGenomics

In Feature Extraction for CytoGenomics, open the InnoScan image that failed in the CytoGenomics workflow.

1 With the Feature Extraction for CytoGenomics main window open, click **File > Open > Image**, and browse to the folder that contains the image file.

2 Double-click the image file (.tif) that you want to open.

Alternatively, you can “drag and drop” the image from your folder to the open Feature Extraction window.

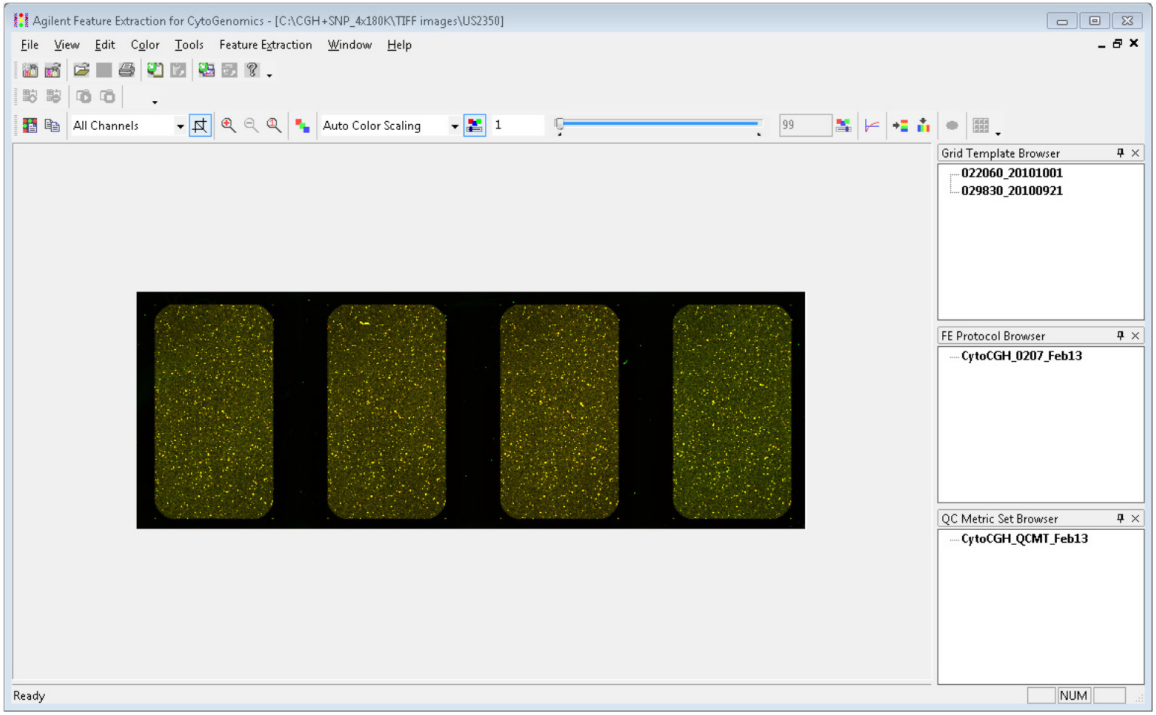


Figure 6 Feature Extraction for CytoGenomics – Main window with image file opened

NOTE

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

Manually grid the image in Feature Extraction for CytoGenomics

Refer to the *Feature Extraction 11.5 User Guide*, Section 3 – "Creating Grid Files: If Automatic Gridding for an Agilent Image Fails" – for instructions on manual gridding. 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 Section 2, "Extracting Microarrays Automatically: To display the Project Run Summary Report" for details on summary reports.

If gridding fails, use the following steps 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. Adjust rotation and skew of subgrids.

Once the extraction is complete after manual gridding, Feature Extraction for CytoGenomics generates a text output to the “FE Common Extracts” folder. You can then use the text file to rerun the workflow in CytoGenomics. See “Rerunning a failed workflow in CytoGenomics” on page 12.

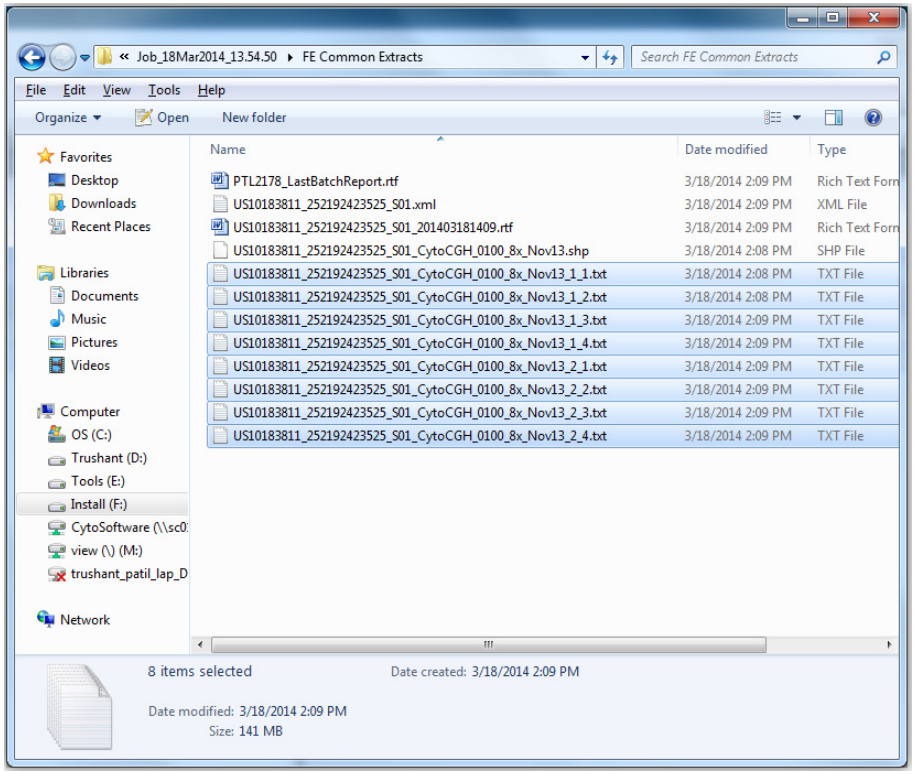


Figure 7 Output text files generated by Feature Extraction for CytoGenomics

Rerunning a failed workflow in CytoGenomics

Once you have the text output file generated by Feature Extraction for CytoGenomics, you can rerun the workflow in CytoGenomics using the text file.

1 On the CytoGenomics Analysis Workflow screen, click **Select Samples** and select the extracted text file (*.txt) as the input instead of a tiff image.

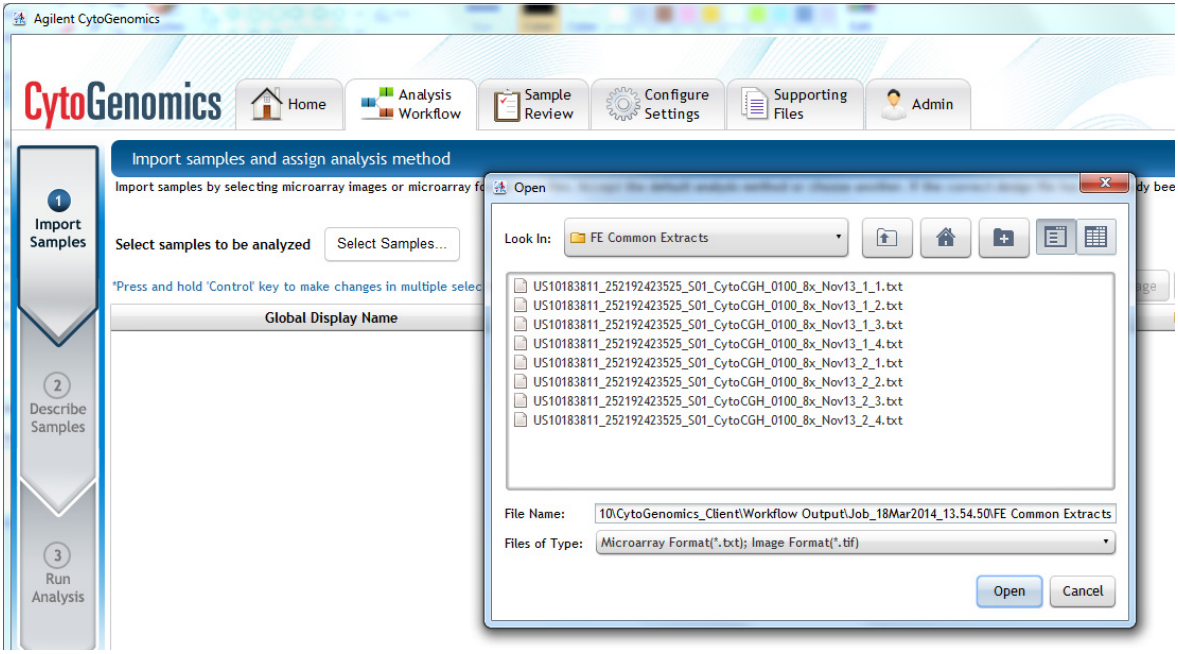
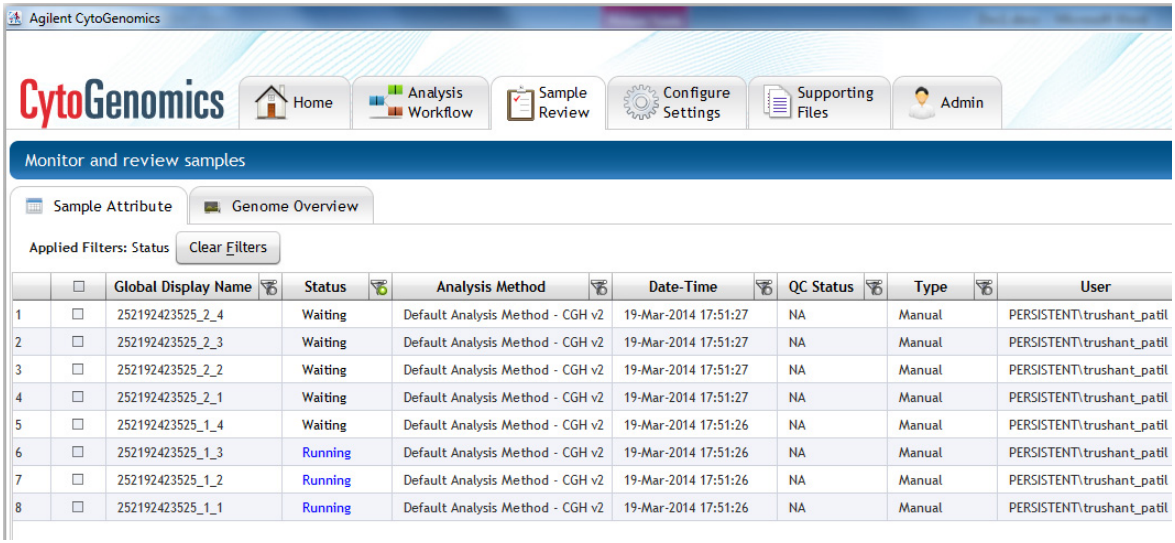


Figure 8 CytoGenomics Analysis Workflow screen – Select Feature Extraction text files

2 Submit the workflow after editing any other attributes for the sample that you want to associate with the array.

The text output files are imported during the workflow run. CytoGenomics can then complete the workflow successfully.



		Global Display Name	Status	Analysis Method	Date-Time	QC Status	Type	User
1	☐	252192423525_2_4	Waiting	Default Analysis Method - CGH v2	19-Mar-2014 17:51:27	NA	Manual	PERSISTENT\trushant_patil
2	☐	252192423525_2_3	Waiting	Default Analysis Method - CGH v2	19-Mar-2014 17:51:27	NA	Manual	PERSISTENT\trushant_patil
3	☐	252192423525_2_2	Waiting	Default Analysis Method - CGH v2	19-Mar-2014 17:51:27	NA	Manual	PERSISTENT\trushant_patil
4	☐	252192423525_2_1	Waiting	Default Analysis Method - CGH v2	19-Mar-2014 17:51:27	NA	Manual	PERSISTENT\trushant_patil
5	☐	252192423525_1_4	Waiting	Default Analysis Method - CGH v2	19-Mar-2014 17:51:26	NA	Manual	PERSISTENT\trushant_patil
6	☐	252192423525_1_3	Running	Default Analysis Method - CGH v2	19-Mar-2014 17:51:26	NA	Manual	PERSISTENT\trushant_patil
7	☐	252192423525_1_2	Running	Default Analysis Method - CGH v2	19-Mar-2014 17:51:26	NA	Manual	PERSISTENT\trushant_patil
8	☐	252192423525_1_1	Running	Default Analysis Method - CGH v2	19-Mar-2014 17:51:26	NA	Manual	PERSISTENT\trushant_patil

Figure 9 CytoGenomics – Sample Review screen

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