



# Agilent Feature Extraction Software Automated Image Analysis Paired with QC Tools

## Product Note

“Fantastic tool! The QC Tool and Metric Sets have enabled us to improve our microarray protocols and ensure optimal quality. It has become an essential component of our microarray processing pipeline.”

—Dr. Alan A. Dombkowski  
Assistant Professor and Director,  
Microarray & Bioinformatics  
Facility Core  
Wayne State University

The Application Note titled “Enhanced Quality Assessment Using Agilent Feature Extraction QC Metric Sets, Thresholds, and Charting Tools” (5989-5952EN) overviews new quality control outputs and tools — metric sets, thresholds, and the QC tool — for Agilent Feature Extraction Software. It is available for download at: [www.agilent.com/chem/fe4me](http://www.agilent.com/chem/fe4me)

One of the big challenges in microarray data analysis is generating reliable, high-quality image-analysis results. Agilent’s Feature Extraction (FE) software reads and processes up to 100 raw microarray image files in an automated, walkaway mode. It finds and places microarray grids, rejects outlier pixels, accurately determines feature intensities and ratios, flags outlier features, and calculates statistical confidences. Application-specific QC reports summarize multiple analyses. FE is a key component of Agilent’s comprehensive informatics platform that integrates complementary technologies and multidisciplinary approaches. It produces output files compatible with Agilent’s GeneSpring GX, GeneSpring Workgroup, and DNA Analytics software, as well as Rosetta® Resolver and other microarray data analysis applications.

### Features-at-a-Glance

- **Extended dynamic range** – Quantify low-abundance transcripts without saturating high-abundance transcripts (using an Agilent scanner)
- **Multiple application usage** – Analyze gene expression (GE), CGH, miRNA, and ChIP-on-chip data
- **Multiple format and density compatibility** – Process both 244K and Agilent’s new SurePrint G3 density arrays as well as 1, 2, 4, and 8 microarrays per slide with the click of the mouse.
- **Output file compatibility** – Analyze with Agilent integrated informatics or public domain programs
- **Convenient yet flexible workflows** – Take advantage of preloaded, user-modifiable default protocols already optimized for individual applications
- **Integration with eArray** – automatically update microarray annotation and image analysis protocols
- **Improved gridding** – Automatically grid single and multiplex microarrays with minimal manual intervention
- **QC Charting Tools** – Efficiently monitor microarray processing performance of hundreds of extractions and create new metrics and thresholds for your own workflow
- **QC Metric Sets with thresholds** – Guidance on identifying key metrics and establishing normal metric ranges (for Agilent Gene Expression two-color microarrays)
- **Software updates** – Be automatically notified of new software releases
- **Convenient QC report PDF** – Easily share QC Reports and QC Charts



**Agilent Technologies**

### Accurate Data Extraction

Feature Extraction software delivers the maximum amount of high-quality data from each and every microarray run. When used in conjunction with a high-density Agilent microarray and an Agilent Microarray Scanner, it extends the dynamic range to accurately measure both very high- and very low-intensity features. Feature Extraction identifies the highest quality pixels in each feature for intensity quantitation, flags outlier features, and detects and removes spatial gradients and local backgrounds. Data is normalized using a combined rank consistency filtering with LOWESS intensity normalization. Output data includes summary statistics for each microarray and feature, including initial, intermediate, and final intensity values, optional log ratio values, automated flagging information, statistical confidence values, and Spike-in regressions.

### Application-Specific QC Reports

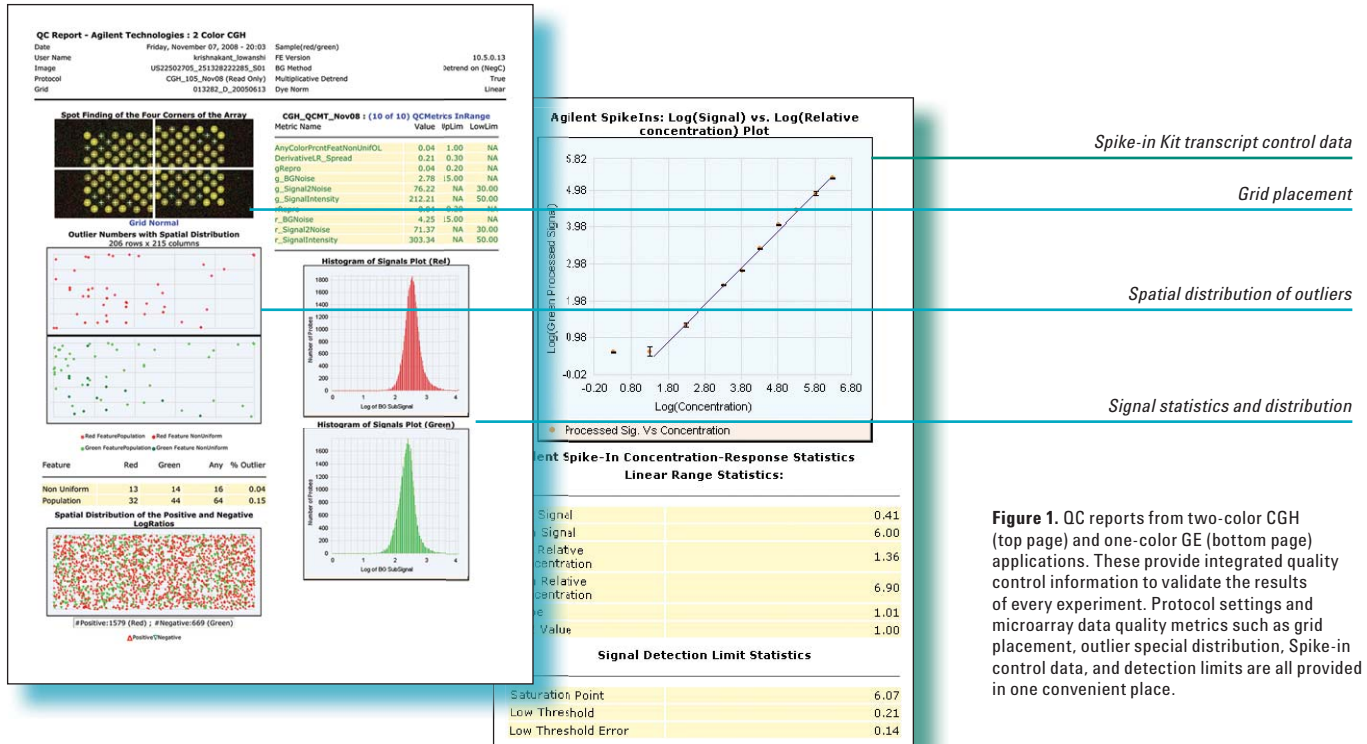
QC reports are tailored to specific microarray applications and document processing quality and reproducibility of sample preparation, labeling, hybridization, and working conditions for gene expression (both one- and two-color) as well as CGH and miRNA applications. A typical QC report like the example shown in Figure 1 contains values and graphs that include general microarray processing metrics, application-specific quality metrics, and Spike-in probe set metrics (for GE platforms).

Built-in QC features detect, disclose, and document data quality issues arising from technical sample preparation and hybridization issues including sample degradation, wash artifacts, and ozone exposure. Feature Extraction reports also provide a visual display of spot finding on all four microarray image corners, and document replicated probes, log ratios, and spatial distributions of statistically significant features.

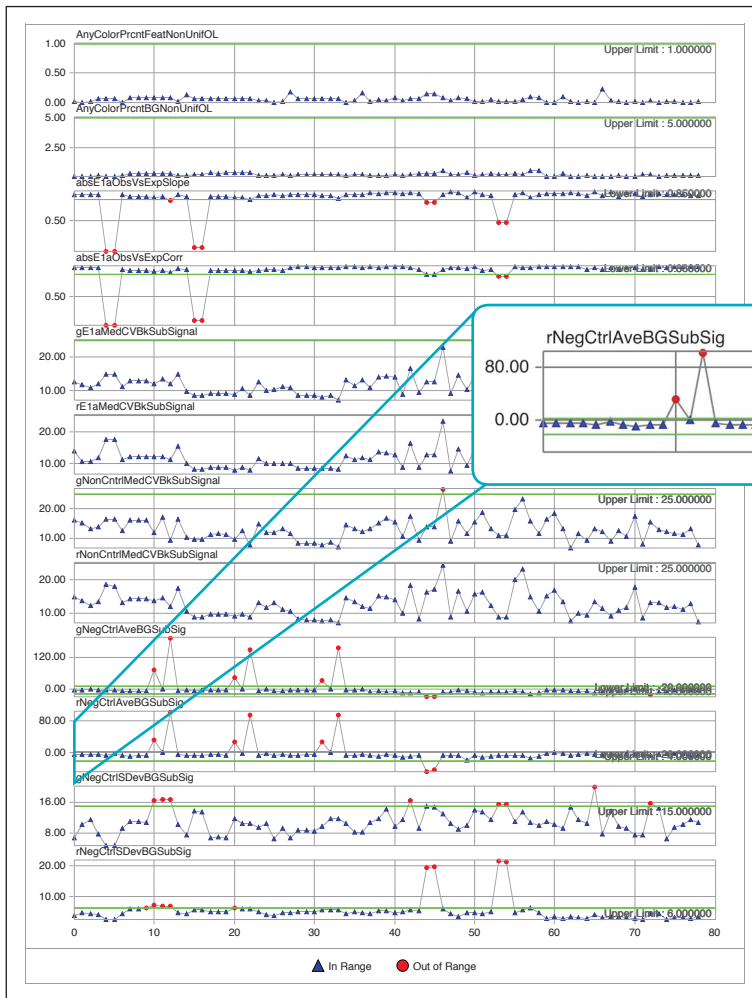
The optional QC Charting tool can generate QC metric sets tailored to your experimental conditions (Figure 2). These metric sets can be imported into Feature Extraction and implemented to monitor processing and overall data quality. It allows customized sorting, color- and shape-by attributes and is a powerful tool for visualizing and highlighting trends and patterns.

### Keep Current with eArray

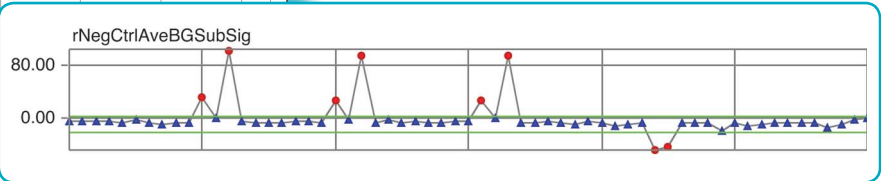
Feature Extraction ensures that you have the latest microarray annotation and image analysis protocols through integration with eArray. Simply input your eArray login information and whenever Feature Extraction runs, it will check for updated information. See Figure 3.



**Figure 1.** QC reports from two-color CGH (top page) and one-color GE (bottom page) applications. These provide integrated quality control information to validate the results of every experiment. Protocol settings and microarray data quality metrics such as grid placement, outlier special distribution, Spike-in control data, and detection limits are all provided in one convenient place.



**Figure 2.** Feature Extractions QC chart confirms the occurrence of processing artifacts and replicate microarray outliers. A metric set containing 12 metrics and thresholds was used on a set of 78 gene expression two-color microarrays extracted by FE. The QC chart shows that several microarrays have more than one metric out of normal range (red circles). Values in range are also displayed (blue triangles). Threshold limits appear in upper right-hand corners and as green lines within each plot. The inset window zooms in on the “rNegCtrlAveBGSubSig” (average of red-channel negative-control background-subtracted signals) metric. Optional vertical gridlines are shown. An accompanying detailed table which maps extraction names to X-axis identifiers is also generated, but not shown here.

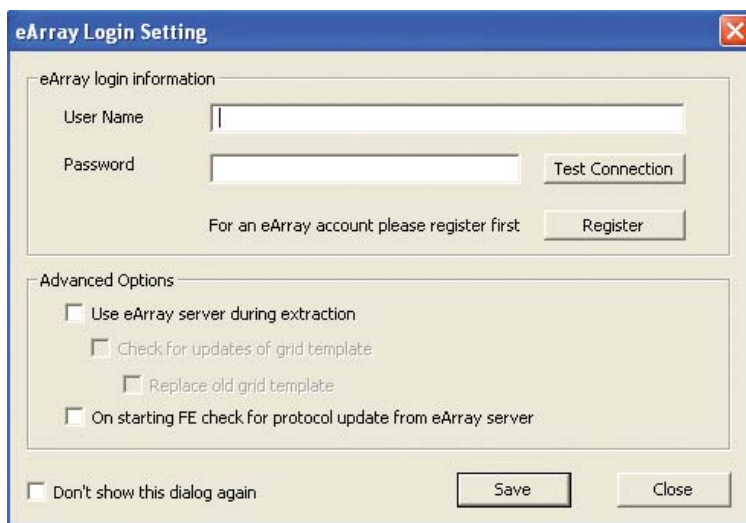


### Efficient Workflow Automation

Feature Extraction has two processing modes for flexibility and user convenience. The “Batch processing” mode automatically reads and analyzes up to 100 loaded microarray images. The microarray design, grid, and protocol annotations associated with each Agilent microarray bar code are automatically paired with the appropriate images. Information from non-Agilent microarrays can be easily entered and assigned through setup screens. The “On-Time Batch” mode interfaces Feature Extraction directly with Agilent SureScan scanners to automatically read and process images as soon as they are generated. Automated FTP uploads save your data to a specified server or workstation.

### Output Files Compatible with Industry Standard Analysis Applications

Images and data are saved in JPEG, GEML, MAGE, tab-delimited text, and other universal file types. Disk space and network bandwidth can be conserved through options to reduce and compress the output files. The data is compatible with a wide variety of informatics packages. The data flow has been tested and optimized for import into Agilent’s integrated informatics products including Agilent’s GeneSpring GX, GeneSpring Workgroup, and DNA Analytics software, as well as Rosetta® Resolver.



**Figure 3.** By entering your e-array login information and selecting the desired Advanced Options, Feature Extraction can automatically check and apply updates to grid templates and extraction protocols.

Microarray Format	Scan Resolution
Agilent High-Density	5 $\mu$
Agilent High-Density Multiplex	5 $\mu$
Non-Agilent	Varies
Agilent SurePrint G3	3 $\mu$

Recommended System Requirements	
CPU	Pentium® III 1.5 GHz or higher (Pentium IV 2.0 GHz or higher recommended)
Operating Systems	Microsoft Windows® XP 32-bit, Windows Server 2003 SP1, Windows Vista 32-bit, Windows Vista X64, Windows XP X64
RAM	2 GB (4 GB recommended for high-density 244K microarrays)
Hard Disk Space	40 GB

PC must be able to load MSDE 2000 Release A version 8.0

Ordering Information	
Feature Extraction Software Commercial 1-year Perpetual Upgrade License*	G4460AA
Feature Extraction Software Commercial 2-year Perpetual Upgrade License	G4461AA
Feature Extraction Software Commercial 3-year Perpetual Upgrade License	G4462AA
Feature Extraction Software Not-for-profit 1-year Perpetual Upgrade License	G4463AA
Feature Extraction Software Not-for-profit 2-year Perpetual Upgrade License	G4464AA
Feature Extraction Software Not-for-profit 3-year Perpetual Upgrade License	G4465AA
Agilent DNA Microarray Scanner Bundle (includes two 1-year Perpetual Upgrade Licenses for FE)	G2565CA

\*A Perpetual Upgrade License is a permanent license that allows for software updates for the specified time period.

The latest version of Feature Extraction is available for download at:  
[www.agilent.com/chem/fedownload](http://www.agilent.com/chem/fedownload)

### About Agilent's Integrated Workflows

Agilent Technologies is a leading supplier of life science research systems that enable scientists to understand complex biological processes, determine disease mechanisms, and speed drug discovery. Engineered for sensitivity, reproducibility, and workflow productivity, Agilent's integrated workflows include instrumentation, microfluidics, software, microarrays, consumables, and services for genomics, proteomics, and metabolomics applications.

Buy online:

[www.agilent.com/chem/store](http://www.agilent.com/chem/store)

Find an Agilent customer center in your country:

[www.agilent.com/chem/contactus](http://www.agilent.com/chem/contactus)

U.S. and Canada

1-800-227-9770

[agilent\\_inquiries@agilent.com](mailto:agilent_inquiries@agilent.com)

Asia Pacific

[adinquiry\\_aplsc@agilent.com](mailto:adinquiry_aplsc@agilent.com)

Europe

[info\\_agilent@agilent.com](mailto:info_agilent@agilent.com)

For Research Use Only.

Not for use in diagnostic procedures.

Information, descriptions, and specifications in this publication are subject to change without notice.

Agilent Technologies shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance or use of this material.

Pentium® is a registered trademark of Intel Corporation

Windows® and Internet Explorer® are U.S. registered trademarks of Microsoft Corporation.

PR7000-0306

© Agilent Technologies, Inc. 2008, 2015, 2016  
Published in the U.S.A. January 4, 2016  
5989-6409EN



**Agilent Technologies**