

Agilent Feature Extraction Software Version 10.7.3.1 (FE 10.7.3)

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G4460-60006 Feature Extraction Software Release Notes

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Product:

- # G2566AA - Feature Extraction provided with the Agilent Scanner
- # G4460AA - G4462AA - Feature Extraction Commercial License
- # G4463AA - G4465AA - Feature Extraction Academic License

This release note contains:

- * Changes in FE 10.7.3.1
- * Changes in FE 10.7.1.1
- * Installation Notes for Feature Extraction Software 10.7.1.1
- * Bug fixes in 10.7.1.1 to known issues
- * Known issues in Feature Extraction Software 10.7.1.1
- * Fixes done in previous versions FE to known issues reported in prior releases

This product includes software developed by the Apache Software Foundation
<http://www.apache.org/>.

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Changes in FE 10.7.3.1

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1. FE 10.7.3.1 is a patch release to address issue with miRNA extractions relating to how the TotalGeneSignal is calculated for some fraction of the miRNAs. The TotalGeneSignal is the sum of the TotalProbeSignals for all probes for the same miRNA. For cases where the "Exclude Non Detected Probes" is set to "True" (the default in FE 10.7.1.1), and there are probes for a miRNA that are not detected, an error occurred where the TotalGeneSignal value from the previous probe group was carried through and added to the TotalGeneSignal. This patch release addresses this issue.

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Changes in FE 10.7.1.1

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1. The FE 10.7 software is an upgrade of any FE 10.x release. The installer handles the upgrade cleanly for all of the different FE 10.x versions. There is no need to uninstall the old version for those running FE 10.x. The installer cleanly upgrades the FE software and database along with the QC ChartTool and database.
2. FE now supports PDF QC reports and PDF run charts for 64 bit Vista and 64 bit XP.
3. The software provides improved grid finding for the new Agilent 30-micron feature size arrays. We have enhanced the current grid finding algorithm to include background peak suppression. The background peak suppression has been enabled for 30u array improving the automatic grid finding rate in our test set. The background peak suppression can be enabled by protocol change for 65u arrays for users have trouble grid finding with

these products.

4. QC metric threshold now have two levels supporting the grading of arrays into 3 categories: excellent, good and poor. The metric set browser has changed to reflect the new threshold treatment and to enhance ease of use and understanding of the metrics.
5. The QC reports have changed to reflect the new thresholds. The table containing the metrics has been updated to show the three grade levels for each metrics with two levels of thresholds. Attention has been given to make it easy for a user to understand why a metric was assigned a given grade.
6. The QC reports have changed to add the saturation value to each and every report. In FE 10.5, QC reports for 20 bit scans did not contain the saturation value so the user could not tell from the report that they were working with a 20-bit scan and what constitutes a signal that could saturate the micro-array scanner.
7. The manual grid finding and fitting process has been made easier for customers.
 - a. There is a help wizard available in the lower left corner of the manual gridding frame that helps guide the user through the process.
 - b. The manual grid finding process has been separated into the third party workflow for those customers scanning and extracting non-Agilent arrays as opposed to those extracting Agilent arrays.
 - i. To manually find and fit a grid to an Agilent image, the user must go into grid mode via the project explorer.
 - ii. To manually find and fit a grid to a Third Party image, the user must go into grid mode via the Grid mode button on the image toolbar.
 - c. There is a new option for adjusting sub grids that allows users to simply pick three corners on the image. The three corners to be picked are the upper left, lower left and upper right. Upon picking these corners the software will automatically adjust the grid for the user.
 - d. The user can enter grid mode even with an edited image, be that a cropped or flipped image. The software will save the image and enter grid mode with the new image.
8. The software provides Spike-In support for the miRNA application FE protocol. The Spike-Ins have been incorporated into the QC report, the tab text output and metrics produced by the software.
9. All new metrics and stats associated with miRNA spikeIns, grid detection, and previously missed stats like PercentileIntensityProcessedSignal have been added to the QC Chart Database.
10. The metric sets have been updated to include two levels of thresholds where known and where appropriate. A new metric has been added to all the metric sets for helping with the detection of grid failures.
11. The software has added new output types for generating the tab text outputs. The software supports now supports FULL, COMPACT, QC, and MINIMAL. Most users will still use the COMPACT output type. Certain customers may choose to use QC or MINIMAL. The columns that are output by these new types are available in the Feature Extraction manual.
12. FE licenses are permanent for a given version. When a user buys the scanner or buys the software they also buy the privilege to upgrade their software for a time period. Usually the time period is one year but it can also be longer. If they upgrade to a new version during that time period, the upgrade is free, and the user can continue to use that new version for as long as they desire. Starting in FE 10.7, the software upon starting, will warn the user that

their ability to upgrade the FE software for free, is about to expire or has expired already. Once their ability to upgrade has expired, a user wishing to upgrade will need to purchase a new license.

13. The software now has the ability to flip and rotate 3u, 2u, and 20bit scans from within the image view. The scans are considered to large to fit into memory so the operation will happen on disk and the transformed image can then be loaded for viewing.

14. Improvements (bug fixes) have been made in the optimize grid fit step. The optimize grid fit step is now better able to adjust grids giving an added improvement to grid finding.

15. Unfortunately, the FeNoWindows output error codes have changed. The changes are documented in the manual under the command line FE section.

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Installation Notes for FE 10.7.1.1
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1. Feature Extraction can either be installed from CDRom or by downloading it from the web site <http://www.agilent.com/chem/fe>.

2. The Feature Extraction 10.7 is an upgrade release of FE 10.x. If FE 10.1, FE 10.2 or FE 10.5 is installed then the FE 10.7 will upgrade your system without necessarily disturbing the grid templates, custom protocols or custom metric sets in your FE database. Also, by the upgrade path the FE 10.7 installer will upgrade your QC Chart database without removing any contents. When launching the installer, the user will be given two options, to upgrade FE or to replace FE. The upgrade path will upgrade both the FE and QC Chart Tool databases, and install the latest versions of FE and QC ChartTool. The Replace FE path will Replace the FE database wiping out all the old grid templates, protocols and metric sets, then it will UPGRADE the QC ChartTool database, and then finally install the new FE and QC ChartTool software. If the prior version of FE is 9.x or earlier the Feature Extraction must be uninstalled before installing Feature Extraction 10.7.1.1. To remove the previous version, go to the add/remove programs option under the control panel and remove "Feature Extraction".

3. The database in Feature Extraction 10.X has changed and is not compatible with previous releases. If you use any special protocols or metric sets, then they must be exported from the current software before uninstalling.

- a. Note that a backup of the QC Chart Tool database prior to 1.3.1.2 release will NOT work with FE 10.7.
- b. If you backup your QC chart database (version prior to the current release with FE 10.7, Version 1.3.1.2) hoping to restore it under FE 10.7, the QC Chart database will be missing the ability to store new key metrics added to support the new Features of FE 10.7.
- c. In this case, you will want to export your data from your old QC Chart Tool and then import it into QC Chart Tool after FE 10.7 is installed.
- d. Users will unfortunately lose their queries as these are not exported but the data can be recovered.
- e. Please see the FE installation guide for more details.

4. Recommended PC configurations for FE 10.7.1.1 are using a Dual Core X86 (please see 5 below) based Processor with 2+ GB of RAM and at least 40 GB of hard disk space. Agilent recommends using 4 GB of Virtual Memory.

5. Agilent has not tested FE on Itanium Processor architectures (IA-64). As such, Agilent does not support Feature Extraction on Itanium based windows machines.

6. The supported Operating Systems are Windows XP Professional SP2, Windows Server 2003 SP1, Vista Business Edition, Vista 64-bit Business Edition, and

Windows XP 64.

7. FE 10.7 has not been extensively tested in Windows XP SP3. If you are wishing to install FE 10.7 on Windows XP SP3, you will likely encounter an installation failure. SQLExpress installation may fail and may require the use of the Windows Installer Cleanup Utility. Use this utility to remove MSXML 6 and then reinstall Feature Extraction. See <http://support.microsoft.com/kb/290301> for details.

8. In order to install FE the user must be a member of the administrator group. Feature Extraction needs to create directories under the "Program Files" system folder and must be able to access and edit the system registry in order to ensure correct installation.

9. On the Vista Operating System both 32 and 64 bit versions, the user must disable User Access Control before installing FE. Not disabling User Access Control (UAC) could cause the software to fail to install as it will not be able to access required system registry to ensure correct installation.

10. After uninstalling the previous version of Feature Extraction, a reboot may be required. If the reboot is requested by the installer and it is not performed then installation of the new version of FE may fail.

11. After the full installation is complete, the installer may ask to reboot the system. The reboot is required and if not done may lead to the software working improperly.

12. FE 10.7.1.1 allows users to run FE with their choice of databases: either SQLExpress <http://www.microsoft.com/sql/editions/express/default.aspx> or MySQL <http://www.mysql.com/>. Feature Extraction uses ODBC to connect to the database but we have built schema for and specifically tested against MySQL. This third party database support was specifically added for those users intending to process large number of arrays with a large number of unique designs.

The SQLExpress database shipped with FE has limits on the number of designs it can hold. MySQL is an inexpensive database that does not have such limitations. Here we list some tips of installing and using FE with MySQL.

a. FE 10.7 uses ODBC to interact with the database, so users installing MySQL need to install the MySQL Server <http://dev.mysql.com/downloads/mysql/5.0.html> and the Connector/ODBC - MySQL ODBC driver

<http://dev.mysql.com/downloads/connector/odbc/3.51.html>. FE 10.7 has been tested against mysql 5.0.51 on windows XP and mysql 5.0.45 on windows Vista. The ODBC tested ODBC driver is Connector/ODBC 3.51.

b. If using MySQL, the user must install the database before installing FE. If you wish to switch to MySQL, then Feature Extraction must be uninstalled and MySQL installed before reinstalling Feature Extraction.

c. Currently Agilent supports using Feature Extraction and a MySQL Server on the same PC. Having FE access a database on a remote machine is not supported.

d. When installing MySQL, please make sure to enable root access from remote machines. This option appears on the MySQL Server instance configuration window when setting the security options. The Feature Extraction software connects via TCP/IP and requires that remote access be enabled.

e. Also, when installing MySQL, please make sure to include the bin directory in windows PATH. This option is settable in the MySQL Server Instance Configuration

Screen. This option is required if you wish to use Feature Extraction's Backup and Restore Utility to manage your database backups.

f. Please contact Agilent Technical Support if you have more questions or problems.

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Bug fixes in 10.7.1.1 to known issues

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Below are known issues that existed in FE 10.5.1.1 that have been fixed in FE 10.7. Some of issues have further explanation if we thought it might be needed.

1. Median Signal not correctly calculated in FE version 10.x for Features above 2^{16} (TT 2197). This issue was related to how the pixels are sorted in order to calculate a median. The sorting issue has been fixed and now the medians are correctly calculated for all features no matter their signal intensity.
2. Show QC chart button does not enable when pdf QC reports and run charts cannot be generated. This means that users will not be able to use the QC chart button on XP/Vista 64 bit (TT 2120).
3. In very rare instances, while running an extraction that fails to properly extract, some messages related to the extraction do not appear in the extraction summary report. The FE extraction fails with a low level runtime error (TT 2099).
4. The user cannot abort the importation of a protocol. The software will hang and will need to be killed using the task manager (TT 2094).
5. Protocols can be saved with a blank protocol name, but such a protocol cannot be used in the software. Adding the blank protocol to an extraction in a project will cause that project to fail to start (TT 2093).
6. Sometimes QC Charts do not get generated at the end of a project even though all the extractions in the project use the same protocol and hence the same metric set (TT 2077).
7. As long as the user reboots after un-install, the following issue will not occur.
 - a. If the user attempts to remove FE via the add/remove programs while the FE instance is open, the FE un-installation fails. The user will need to run the installer to repair FE and try the un-install again with the FE instance closed (TT 2057).
8. Sometimes the software will generate a memory error when try to load shape (.shp) files on high resolution scans. The work around is to close the software and restart it. Then the image and the shape file will be viewable (TT 2026).
9. If the user adds a grid template to the database while in manual grid mode, then certain features of third party manual grid mode can become disabled. Specifically, if the spot mode will become disabled (TT 1969).
10. Sometimes QC Charts are not generated after an extraction run completes. When this happens, the software does not generate a pdf of the run charts of the selected metrics and an error message will appear run summary report. Once receiving the error message, the user will not be able to generate the chart via the menu. The only workaround is to quit and restart the FE software, followed by

running the project again (TT 1925).

11. Sometimes FE is unable to create a grid file for manual gridding when using third party arrays. This will happen when attempting to create a grid file from a tab text file or GAL file and either not enough information exists about the array geometry or the geometry information specified is wrong leaving the software to guess what the correct geometry is. The workaround to this is for the user to manually measure the geometry in pixels on the image before starting grid mode.

The user must measure the spacing between spots in both the horizontal and vertical directions (measured spot center to spot center), the spacing between sub grids in both the horizontal and vertical directions (measured from the last spot in the first sub grid to the first spot in the next sub grid), and the diameter of each spot.

Typing these values into the software if they are incorrect fixes the issue (TT 1908).

12. Data exported to Excel from the Histogram plots or Line plots has the first value corrupted. All other data values after the first are correct. This only happens

on Windows Vista Operating System but may be related to the version of Office being used.

Under windows XP with office 2003 all data values exported to Excel from the plots are correct (TT 1884).

13. Save histogram or line plot value for single channel scan not working (TT 1972).

14. We now report in the run summary that this option is not supported. Users will

get a warning if they try to do this:

- a. Ultra high density Agilent arrays, meaning Agilent arrays containing approximately 976,000 features, scanned at 5 micron scan resolution cannot be reliably processed using Feature Extraction 10.1.1. Memory issues can surface while processing, automatic grid registration is significantly less reliable and Manual grid registration is difficult. We strongly recommend scanning these arrays at 3 micron resolution or higher (TT 1775).

15. We now support the flipping and rotating of 2u, 3u or 20bit scans so this issue below has been fixed:

- a. New format scans (e.g. 2 micron, 3 micron or 20 bit scans) cannot be flipped or rotated. 5 and 10 micron 16 bit scans are in the same format as before and can be flipped and rotated.

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Known Issues in Feature Extraction Software 10.7.1.1 and earlier

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- Left out of the command line chapter, chapter 6 of the FE Reference guide is a warning that has been added to the software. All of the error and warnings the software can produce while extracting an array are listed at the end of that chapter.

The warning we missed says "Agilent does not support this configuration, please consult the support matrix in the Feature Extraction users guide for a supported configuration". The warning ID for this is 1060. Note that when referring users to the users guide, the configuration information is explained on page 19.

- Grid mode save grid does not allow projects to be run. The user has to close grid mode after the grid has been saved before FE will correctly update the project explorer and allow the project to be run (TT 2310).

- Changing the color scale does not work while in grid mode. If the user attempts to change color scale from "All Channels" to "Red Channel" or "Green Channel" it does change (TT 2309).
- The 1-color QC report has page break problems causing one of the pages to break across a table when the QC report is put into PDF mode (TT 2299).
- The older version of easy PDF, easy PDF 5 not uninstalled on upgrade to FE 10.7. This is because we have moved to easy PDF 6 and the upgrade installer doesn't correctly handle uninstalling the old component. Work around is to delete the easy PDF 5 printer directly through Start->Settings->Printers and Faxes. The instance of easy PDF 5 does not create any problems for FE 10.7 extractions (TT 2292).
- Attempting to extract an Axon Scan without a barcode, grid template/grid file or protocol will crash Feature Extraction requiring the machine to reboot (TT 2289).
- Extraction automation does not work for Agilent cropped multiplex images. This is because we adorn the pack information on the barcode so it doesn't get lost when the image is cropped (TT 2287).
- Trying to extract a third party array by putting an Agilent barcode inside the tiff image will lead to the software failing with a low level runtime error (TT 2272).
- Due to rounding errors, a metric value shows "Inrange" in QC chart tool and "Out of range" in FE. This only happens for metrics at the border of the threshold (TT 2261).
- IsGoodGrid value shown inappropriately on run chart. The chart for this has no Y-axis values (TT 2260).
- For metrics that get blank values as assigned by FE, these same metrics inside QC Chart Tool get assigned 0 or 0.0 which is not the same as blank (TT 2243).
- The eArray integration feature is not quite complete in that after FE runs, the project explorer is not updated to reflect which grid template and protocol was automatically assigned. This can be confusing to a user after the project has finished as they do not get the feedback in the Project Explorer (TT 2236).
- Sometimes for arrays from designs that have high numbers of ignore features the user will get "INFO: (Array_1_1) This array doesn't have any found spots. Maybe the array has not been hybridized." The calculation that generates this message is not correct (TT 2227).
- Flip functionality fails with a poor error message if not enough disk space is available to complete the operation. The error message reads "Save as failed due to unknown reasons". (TT 2210)
- For CGH, and GE2 QC reports, the log ratio spatial plot has errors in sampling. Currently, if there are > 3500 significant Up or Down-regulated log ratios, then the code does a sampling. This leads to a confusing appearance of the number of up and down regulated features (TT 2207).
- After a scan is generated, the image should retain the parameters by which it

was generated, and they should be retrievable from the Feature Extraction menu item Image File Info..., which displays the Scan Image Properties window. Parameters that are currently missing include:

- Single pass or Double pass
- 16-bit or 20-bit
- Laser set points and/or actual laser power readings for each channel (this should reflect compensation, if invoked)
- PMT compensation factor
- PMT Calibration Error flag status (TT 2203)
- For new format scans, on 64 bit OS PCs, the first submenu option i.e "Flip upper left to lower right" (Landscape/portrait) and save" is not completely shown (TT 2190).
- FE 10.7 does not support MySQL version 5.1. If you have MySQL version 5.1, FE may fail to install (TT 2188).
- During installation of FE, FE has to install the java runtime. During that installation, a dialog box "Copying JRE files" is displayed with a cancel button. Clicking the cancel button will corrupt the FE installation. The Box should be hidden (TT 2187).
- QC report contents can appear broken in that tables and graphs don't fit on to the pages all that well. The workaround is to adjust the printing margins in internet explorer if the user is using I.E. 6.0. We have not seen the issue under IE 7 or later (TT 2179).
- FE eArray automation. Issue: customers are confused by the red "X" that is displayed in FE and don't realize that FE can operate without a protocol assigned and feel more comfortable if they know what protocol will be used (TT 2175).
- For miRNA applications sometime you can get some weird looking %CV of the replicated probes plot on the miRNA QC Report if there are no CVs to plot. This can happen if we can not calculate any replicate %CVs because the data is not good enough (TT 2171).
- For new format scans while setting color ranges, the ability to adjust colors in high fidelity mode can lead to errors – namely if the user tries to set "Auto Scale Image" after unsetting this option. (TT 2166).
- Find feature not working for third party arrays (TT 2149).
- If a user attempts to import a grid template during an extraction run, the grid template may fail to properly import into the software. The work around is to only import grid templates when a project is not running. (TT 2112).
- When importing text data into the QC Chart tool generated with the new CGH protocol supporting and generating Streamlined CGH QC reports or the miRNA protocol, the QC Report type column is blank in the QC Chart Database. The QC Report Type column is correctly loaded for the GE1, GE2, old style CGH, and the ChIP QC Reports (TT 2109, 1724).
- Attempt to abort an extraction on Vista 64 will cause the extraction to stop ungracefully with a low level runtime error (TT 2092).
- The eArray automatic online protocol update can fail if either the

protocol

or the metric associated with that protocol are missing (but not both) when the update occurs.

It is possible to make this happen because even read only protocols and metric sets can be

removed using FeNoWindows. The worst case is if the metric set is missing from FE, then the

eArray will fail to update the protocol due to a missing metric set (TT 2075).

- Sometimes in rare instances, the switch to configure mode button will not be enabled after the extraction is complete. The extraction will have to be closed and re-opened to enable to config / run mode toggle (TT 2069).

- The grid template that is currently in use during an extraction, can be removed causing the extraction to fail (TT 2042).

- The eArray configuration setting "Automate FE extraction by automatically downloading design files from eArray" can cause unwanted behavior if the user wishes to extract an image using a grid template with an AMADID doesn't match the image's AMADID. Disabling the eArray configuration setting temporarily causes FE to behave correctly in this case (TT 2040).

- Attempting to calculate spot size and centroids in manual grid mode using a high resolution scan of a third party array will cause FE to crash (TT 2032).

- In rare instances the QC report can fail to generate. This is true when 30um feature size 2-pack arrays are used in FE, using a CGH protocol that generates an old style CGH QC report. For the 2-pack 30-micron feature size arrays the new streamlined CGH QC report must be used (TT 2019).

- It is not recommended to run projects containing multiple extractions directly through FeNoWindows. Projects containing multiple 30-micron feature size 1 million feature array will run out of memory if run directly using FeNoWindows. The work around is to either use the FE GUI to run these projects or to break up the project into multiple projects each containing 1 extraction (TT 2016).

- Do not remove the DBConnectInfo.ini file from the FE installation folder. If that file is not available the software cannot be removed via the install or Add/Remove programs (TT 1944).

- When viewing shape files, feature outliers are not visible until the image is zoomed or cropped (which effectively zooms) (TT 1955).

- Viewing the scan properties can cause the image to appear distorted. Minimizing followed by reopening the image will correct the issue (TT 1959).

- Sometimes FE goes in to not responding state after clicking on "View QC Chart". This error is sporadic and is only seen after multiple extractions are done (TT 1925).
- Scans of 2, 3 and even 5 micron resolution using full sized scan regions are quite large creating memory issues for the software. In order to address memory and performance issues the following restrictions are true about the imaging.
 - a. The new view window feature (with Ctrl-N as the shortcut) that allows users to open one channel of the scan will not work for new format scans.
 - b. When cropping a new format scan to view the image close up, what FE refers to as high fidelity mode, zoom out is disabled below 200%.
- When creating a grid file of Agilent arrays some annotation columns may be lost. Currently only the primary annotation columns are certain to make it into the grid file. Those annotation columns are Probe Name, Systematic Name, and Gene Name. Other annotation fields are not certain to be output. There is no workaround to this issue (TT 1917).
- For large grid templates using the dye normalization list editor can take a very long time. This is a function of the number of probes that have to read from the database and loaded into the editor. It is possible to create the lists outside of FE and load them into the software without using the list editor. Please consult the FE manual for details (TT 1913).
- Blank fields, namely blank annotation fields above and beyond the primary annotation fields of Probe Name, Systematic Name, Gene Name, used inside of the GEML xml design files will fail to load into the MySQL database. The MSSQL database is unaffected by blank annotation fields. The workaround is to load minimally annotated designs or to get updated designs via Agilent support (TT 1912 and 1853).
- Changing passwords in the database server will break the database connection for FE. For MySQL the root server password provided when FE is installed must be maintained. For MSSQL/SQLExpress the password must be "#Welcome\$" (TT 1859).
- Under Windows Vista, FE users may not have permission to write to certain directories or overwrite certain files. This will cause FE to fail to load grid templates, protocols and/or metric sets. Also it will cause FE to fail to process extractions. The solution to this is to make sure the User Access Controls are turned off and ensure that C:\Program Files\Agilent\MicroArray\FE\Temp is writable and empty (TT 1836).
- It is possible using the FE to remove a grid template while the software is processing extractions. This is not recommended as it could cause the project to fail if that grid template is used (TT 1826).
- Applying many different image manipulations including Flip and Rotate on 5

micron 16-bit scans may cause FE to run out of memory and yield the message "Failed to load bitmap". The workaround is to close the FE software re-open it and try the operation again (TT 1874, 1768, and 1766).

- Protocols that are loaded into the database by name are specifically not case sensitive but protocols specified in the project are case sensitive. Please keep all protocol names the same case (e.g. stick with capital letters) (TT 1699).
- When cropping an image using the new crop image dialog, the file name is appended with 1_1 although "Cropped multiplex" is not selected (TT 1679).
- When running an XDR extraction project, the summary report can give grid placement error '... The spot centers are shifted relative to their nominal grid.' But the QC report says the grid is normal. This is an indication that XDR failed to properly extract the low PMT scan and there may be an issue with the image registration (TT 1675).
- When using FE 10.1.1.1 with PDF QC reports and charts, sometimes a blank QC Chart is generated after extraction. Rerunning the project again will generate a corrected QC Chart. This is directly related to having Internet Explorer (IE) open when running Feature Extraction. We recommend not using IE while running FE (TT 1667).
- When a two-channel tiff file is split into two single-channel tiff files (one for Red and one for Green) from the Agilent Microarray Scanner, On-Time project treats the split tiff files as two separate single-channel files (TT 282).
- Protocol, DyeNormList, or GridTemplate cannot have special characters in the name or description. The character that is sure to break is"" (TT 652 & 657).
- FE is not designed to support concurrent users. The software will only allow one user to use FE at a time. If another user is logged into the same machine, only one will be allowed to run FE.
- You cannot open a .tif image from a CDRom or DVD. Copy the image to a hard disk or network share.

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Fixes done in previous versions FE to known issues reported in prior releases

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Fixed in FE 10.5.1

- In the FE 10.2 prerelease version, QC reports for the 30 micron feature size 2-pack arrays fail to generate QC reports. This issue has been partially fixed. The FE 10.5 release generates by default a new streamlined CGH QC report. This new QC report generates in all cases tested (TT 2019).
- When viewing images using the Feature Extraction GUI, 5 micron scans take more memory than in FE 9.5.3 (TT 1874).

- Sometimes when saving the image as a JPEG generates no JPEG and no error message is generated. The workaround to this issue is to restart the software and try saving the image as a JPEG again (TT 1920).
- In order to speed up the Viewing of shape files, the software now by default turns on the option of viewing outliers only. This greatly improves performance when loading the shape file. The users will need to crop a region of the image and then turn off view outliers only under the Extraction Results menu in order to view all shapes (TT 1942).
- Some designs could not be loaded into FE 10.1.1 or earlier release because the annotation contained in the designs were too long for the FE database. This has been fixed in FE 10.2 (TT 1943).
- When working with dye norm lists in FE 10, only external dye norm would work correctly. Dye norm lists attached directly to a grid template could not be loaded in to the FE database. This has been partially corrected but the software still does not allow over 50000 probes to be loaded and attached to a grid template (TT 1948).
- In FE 10.1, using dye normalization lists in Compact mode caused FE to stop not completing the processing of an array. The software would error with 'low level runtime error'. This issue is fixed in FE 10.2 (TT 1949).
- In the FE 10.2 pre-release the new option allowing users to view only pixels used in the signal calculation, is limited to those features that are completely contained inside the cropped region. Any feature only partially inside the cropped region will have all of its pixels turned off (TT 1952).
- The grid geometry dialog box did not accept enough digits of precision to modify the spot spacing the new 1 Million feature arrays. The new dialog allows for more precise entries (TT 1953).
- After upgrading FE, if the user may see 2 FE shortcuts. This happens if the reboot option is not chosen. The old shortcut has been removed but the desktop is not reflecting this. Having windows refresh the desktop fixes this issue (TT 1965).
- When generating a MAGE-ML file using FE 10 can cause the MAGE-ML file to have slightly incorrect information. More specifically, the laser power value, unused by the scanner, is set to a garbage value. The FE 10 software writes the laser power value out into MAGE-ML output file and can write an un-initialized value causing the Rosetta Resolver to fail to load those effected MAGE-ML files. This has been address in FE 10.2/FE 10.5 (TT 1970).
- Given that we automatically assign protocols, this bug should not occur: Using the wrong protocol to extract a high density or ultra high density arrays can cause Feature Extraction to process for a very long time. This will happen if you attempt to use the CGH protocol on arrays with large numbers of negative control features (TT 1902).
- Sometimes opening perfectly valid scans in the FE GUI for image viewing fails with an error message like 'An invalid file handle was associated with file.tif'. Attempting to open the scan again will succeed (TT 1788).
- When installing FE the QC Metric set browser is not shown by default. The user must go to the view QC Metric Set Brower menu in order to get it to display (TT 1674).

Fixed in FE 10.1.1

- 1 color Axon scans cannot be opened or processed in FE (TT 1740).
- FE allows extraction to start without selecting the output folder. If the user opens a new standard project and in the project properties tab on the local file folder selects "same as image" as false and doesn't enter a results folder FE will crash after processing the image or images (TT 1739).
- Running a legacy background subtraction algorithm called "Global Background Adjust" fails for multipack arrays (TT1732).
- Installer fails on Vista 64. SQL Express fails to install due to the path to the database being too long. The FE installer now correctly constructs a path to support the SQL Express database installation (TT 1728).
- SQL Express fails to install properly on systems without Windows installer 3.1 installed and/or MDAC version 2.8 installed (TT 1727).
- "FTP send PDF only" option does not work in project settings. Attempting to ftp PDF QC Reports yields the message "FTP of QC Report is not supported in 9.1" and the reports are not transferred (TT 1726).
- Auto estimate additive error not possible for 3rd party arrays (TT 1721).
- Spatial distribution plots incorrect drawn. For 244k CGH arrays the spatial distribution of significantly positive and significantly negative probes on the QC report is incorrect. The top half of the plot contains significantly more points than the bottom half. This is an issue with the plot and not the data generated by FE (TT 1706).
- When running a project that contains XDR extractions, sometimes the project will fail with a low-level runtime error. This is because FE failed to allocate memory to read the low-pmt scan. This is a workaround to a more serious issue part of FE 9.1 and 9.5 causing the generation of bad data. This issue is very infrequent but if it does occur you need to exit the application and restart before running your project again (TT 1705).
- Feature Extraction 9.5.3 can run with multiple threads (FE 10 runs only 1 thread). With the high density Agilent arrays (scanned at 5 microns) it is possible for a thread to stop running due to lack of memory. When this occurs the extraction being run in that thread re-enters the queue and gets processed by another thread. When this occurs, it is best to let the project complete successfully and then close and re-open the Feature Extraction SW before running additional projects. If enough projects are run without closing the Feature Extraction SW, occasionally even the final thread will fail to complete due to lack of memory or the application will stop responding (TT 1489).

Fixed in 9.5.3

- Loading multi-file tiffs can assign the wrong channel. For example pick any 2-color tiff (GenePix or Agilent) spread across 2 files. Opening the Green channel file first will load the green image into channel 0. Opening the red channel tiff first will load the red image into channel 0. If the green channel is channel 0 and the image is processed then the channels will be flipped in the output file (TT 1716).
- The use of Dye Normalization lists is broken for multipack array processing. If the user attempts to use a dye normalization list with a multipack array the software will run out of memory or encounter a low level runtime error (TT 1704).

- Multiplicative Detrending runs on the wrong class of controls causing the algorithm to consider very few true replicates (in the case of custom designed Agilent microarrays). The effects of this can be quite different than what we would expect (TT 1701).
- For 1-Color scans, Image properties dialog displays incorrect information on Scanner offset section of the info dialog (TT 1670).
- MAGE-ML outputs for arrays processed with 1-color protocols and 2-color protocols which calculate the error model directly after background subtraction do not include the error model parameters, relying on Rosetta Resolver's defaults for Multiplicative and Additive Error terms. The 2-color protocols in question here were introduced with FE version 9.1 of the software and have '91' in their name. This leads to large differences in significance calls between the Feature View of a single array and any combined view in Resolver (Reporter view, Gene view, any multiple array view). 2-color protocols which calculate the error model after the ratios are calculated, correctly output Agilent's error model parameters into the MAGE-ML file for use in Resolver and do not display this problem (TT 1654).
- The software generated invalid MAGE-ML when a security group is not specified in the project. The security group and owner were meant to be optional so the software should have generated valid MAGE-ML without adding this (TT 1634).
- If a user chooses to output grid file from a multi pack array, then uses that grid file to extract, the results are wrong (spot find shows all sub-arrays pointing to 1st sub-array position). Stats for sub-array 2, 3, 4 are identical (TT 1631).
- The BGUsed column of FE is not correctly calculated when additive detrending is applied with another background method (TT 1629).
- When cropping an Image, the image crop and save doesn't save the area cropped by the user. The software saves a different area not related to the cropped area (TT 1618).
- Background Subtraction: The Global Background adjustment is not calculated correctly. The adjustment appears to be subtracting double the calculated value. Global background adjust is turned off by default for all Agilent recommended protocols and we do not currently recommend its use (TT 1610).

Fixed in 9.5.1

- Negative control standard deviation as calculated is not robust. This can be seen with CGH arrays that have "hot" negative control features. These features are artificially and locally high due to foreign substances on the array surface and are therefore candidates to be rejected as outliers. Normally FE would take care of this via the population outlier rejection but for CGH arrays the populations of negative controls are often smaller than minimum number of features needed for population outlier rejection. This can negatively affect downstream calculations (TT 1616).
- European users of FE with annual licenses can get the message 'License will expire in -38952 days'. This is because we don't correctly recognize the European date format (TT 1615).
- The Run chart key of run charts viewed after the completion of an FE batch

do not display pack information about the barcodes in the run chart making it impossible to distinguish which array a chart value refers to (TT 1614).

- The local background subtraction option is completely disabled in FE 9.1 and FE 9.1.3. Enabling local background subtraction in the protocol and then processing arrays with that protocol yield no difference in background subtracted signals due to local background (TT 1612).
- Image manipulation functions, flip and rotate image are not working after loading only green channel image (TT 1599).
- Processing of split and rotated tiff images through FE 9.1 and FE 9.1.3 is not possible, but was previously possible in FE 8.5. Attempting to process split and rotated tiff images yields an error from the software.
- On the QC Report when using a metric set, metric without thresholds were being evaluated and always showing a red 'evaluate' designation. Since there is no threshold, they should show as normal (TT 1591).
- QC Report doesn't correctly position *** for SpikeIn Table (TT 1588).
- Multipack arrays manually gridded then processed through FE yield strange results, the 1st array is OK all arrays after this array have double entries in the SpikeIn table. The SpikeIn plot is also affected (TT 1584).
- By selecting compact text mode as an output type along with MAGE-ML generation yields a different and incorrect MAGE-ML output file. Selecting full text mode yields a correct MAGE-ML file. Compact tab text output should have no effect on MAGE-ML whatsoever (TT 1571).
- The Red Multiplicative Error Value is incorrectly set for the GE2_NonAgilent_91 protocol (TT 1570).
- The row and column information is incorrectly output on 3rd party arrays. The issue affects the QC report as well causing the outlier plots and the up and down regulated plots to become scrambled (TT 1555).
- In the 1-color QC Report, the spike in detection limit in the "Spike-In Concentration-Response Statistics" section incorrectly shows -1, instead of real number (TT 1484).
- Feature Extraction compact output does not include 2 parameter fields that are very useful and desirable in the QC Chart tool. The columns are FeatureExtractor_ColorMode and FeatureExtractor_QCReportType (TT 1648).
- The percent GeneNonUniform Outliers in the stats table and in the QC Report is incorrectly calculated for two color microarrays. The percentage is about 1/2 of what it should be.
- Feature Extraction can now be installed on windows Vista OS and on 64-bit operating systems for X64 architecture machines.