Release Note

Agilent Feature Extraction Software Version 10.1.1.1 (FE 10.1.1)

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G2566AA Feature Extraction Software Release Note
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Product:
G4460AA - G4462AA - Feature Extraction Commercial License
G4463AA - G4465AA - Feature Extraction Academic License

This release note contains:
- Installation Notes for Feature Extraction Software 10.1.1.1
- Known issues in Feature Extraction Software 10.1.1.1
- Fixes to known issues in Feature Extraction Software 9.5.1, 9.1.3 and 9.1.1

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

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Installation Notes for FE 10.1.1.1
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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 is not an upgrade release. The prior version of Feature Extraction must be uninstalled before installing Feature Extraction 10.1.1.1. 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 has changed and is not compatible with previous releases. If you use any special protocols or metric sets they must be exported from the current software before uninstalling. Database backups are from FE version 8.x, or 9.x are not supported in FE 10.
4. Recommended PC configurations for FE 10.1.1.1 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 or greater (please see 7 below), Windows Server 2003 SP1 or greater (please see 7 below), Vista Professional (please see 7 and 8 below), Vista 64-bit Professional (please see 7, 8, and 9 below), and Windows XP 64 (please see 7 and 9 below).
7. 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.
8. 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.
9. Current PDF QC report generation does not work in Vista 64 and XP 64. Agilent is working to solve this issue and will have a patch to FE to enable this support when appropriate drivers are available to us.
10. Until full installation is complete, the system may need to be rebooted a few times. It is required that the same user with administrative privilege logs in after each reboot to complete the installation. Otherwise, accessing Feature Extraction may cause problems.
11. FE 10.1.1.1 has a new feature that allows users to run FE with their choice of databases: either SQLExpress http://www.microsoft.com/sql/editions/express/default.mspx 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 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 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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Known Issues in Feature Extraction Software 10.1.1.1
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- 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.
    o 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.
    o 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.
    o Zooming in on an image disables grid mode for all scans. Grid mode can only be started from a freshly opened scan.
    o When cropping a new format scan to view the image close up, what FE refers to as high fidelity mode, zoom out is disabled.
- 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 (SCR 1925)
- 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 (SCR 1920).
- When writing 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

ProbeName, Systematic Name, and Gene Name. Other annotation fields are not certain to be output. There is no workaround to this issue (SCR 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 (PVCS 1913).
- Blank fields, namely blank annotation fields above and beyond the primary annotation fields of ProbeName, 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 (SCRs 1912 and 1853).
- Sometimes FE is unable to a create 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 use 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 (SCR 1908).
- 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 (SCR 1902).
- 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 (SCR 1884).
- When viewing images using the Feature Extraction GUI, 5 micron scans take more memory than in FE 9.5.3. The total number of 5 micron 16 bit images that can be view at a time is 1. FE 9.5.3 could load up to 4 5 micron scans simultaneously (SCR 1874).
- 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$" (SCR 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 (SCR 1836).

- It is possible using the command line 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 (PVCS 1826).

- 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 (SCR 1788).

- 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 (SCR 1775).

- 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 (SCR 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) (SCR 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 (SCR 1679).

- When running a XDR extraction project, summary has given proper gridding error as '(Array_2_2) the grid may be placed incorrectly. The spot centers are shifted relative to their nominal grid.' 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 (SCR 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 (SCR 1667).

- 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 (SCR 1674).

- 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 (SCR 282).

- Protocol, DyeNormList, or GridTemplate cannot have special characters in the name or description. The character that is sure to break is """(SCR 652 & 657).
- FE is not designed to support concurrent users. The software will only allow one user to user FE at a time. If another user is logged into the same machine, only one will be allowed to run FE.
- You can not open a .tif image from a CDROM or DVD. Copy the image to a hard disk or network share.

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Known issues in Previous Versions fixed in 10.1.1.1
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- **Fixed in FE 10.1.1**
  - 1 color Axon scans cannot be opened or processed in FE (SCR 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 (SCR 1739).
  - Running a legacy background subtraction algorithm called "Global Background Adjust" fails for multipack arrays (SCR 1732).
  - 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 (SCR 1728).
  - SQL Express fails to install properly on systems without Windows installer 3.1 installed and/or MDAC version 2.8 installed (SCR 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 (SCR 1726).
  - Auto estimate additive error not possible for 3rd party arrays (SCR 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 (SCR 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 (SCR 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 (SCR 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 (SCR 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 (SCR 1704).
  - Demo license now gives the proper warning message when it is about to expire (SCR 1702).
  - Multiplicative Detrending runs on the wrong class of controls causing the algorithm so consider very few true replicates (in the case of custom designed Agilent microarrays). The effects of this can be quite different that what we would expect (SCR 1701).
  - For 1-Color scans, Image properties dialog displays incorrect information on Scanner offset section of the info dialog (SCR 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 (SCR 1654).

- o 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 (SCR 1634).
  - o 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 (SCR 1631).
  - o The BGUsed column of FE is not correctly calculated when additive detrending is applied with another background method (SCR 1629).
  - o 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 (SCR 1618).
  - o Background Subtraction: The Global Background adjusts 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 (SCR 1610).
- **Fixed in 9.5.1**
  - o 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 are 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 (SCR 1616).
  - o 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 (SCR 1615).
  - o 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 (SCR 1614).
  - o The local background subtraction option is completely disabled in FE 9.1and 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 (SCR 1612).
  - o Image manipulation functions, flip and rotate image are not working after loading only green channel image (SCR 1599).
  - o 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 (SCR 1591).
- QC Report doesn't correctly position *** for SpikeIn Table (SCR 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 (SCR 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 (SCR 1571).
- The Red Multiplicative Error Value is incorrectly set for the GE2_NonAgilent_91 protocol (SCR 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 (SCR 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 (SCR 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 (SCR 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.