IN Cell Analyzer

Acquisition Software, Version 4.5

Release Notes

March 11, 2014

Features

- Redesigned the user interface:
  - Floating windows now consolidated to a fixed panel layout with adjustable panel boundaries.
  - Added a Dashboard at the top of the Protocol Designer that replaces the Wavelength Chooser for simple instrument control and also allows for quick changes to key protocol settings.
  - Simplified the protocol setup and acquisition workflows.
- Added the following option to the main program configuration file (***IN Cell Analyzer 2200.xml, IN Cell Analyzer 6000.xml, or IN Cell Analyzer 2000.xml***) to allow images to be saved directly to a network drive (without first saving the images to the local drive).

  ```
  <save_to_network>
   false
  </save_to_network>
  ```

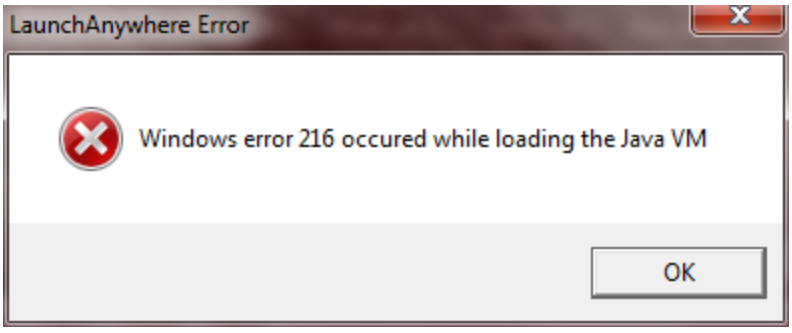
  The site administrator must add this section to the file to activate the feature. By default, the software will spool images to the local disk before copying files to a network drive. After making changes and saving the file, you must restart the user interface.

Improvements

- In the ***Data Review*** mode, you can now:
  - Open single *TIFF*-format image files
  - View images from multiple wells or multiple channels side-by-side
  - View scan results with the heat map feature; results may be exported as a CSV file
  - View hardware and software autofocus data for failure analysis.
- Added ability to export information from image stacks (XDCE files) to CSV files.
- Updated the ***Wavelength Manager*** to allow the display of system (pre-defined) wavelengths and to define and save custom wavelength combinations.
- On the main toolbar, added an ***Apply Offset*** checkbox to the HWAF and SWAF controls.
- Implemented various improvements related to the correction collar (SAC) initialization and control.
- Enhanced the "***Wells-to-cells"*** capability by adding measurement options to ***Review Scan***.
- Changed exposure and Z offset increments.
- Added ability to view the measured hardware autofocus peak separation to the user interface.
- Increased throughput for shorter scan times.

Installation Notes

Installation packages are named according to the instrument model. Be sure to choose the installation package that matches the instrument. Look for "2000", "2200", or "6000". For workstations running 64-bit Windows7, use installation packages that contain "x64" in the file name. Packages that do not contain "x64" in the file name are intended for workstations running 32-bit Windows (XP or Windows7). Attempting to install the 64-bit package on a workstation running a 32-bit operating system will result in the error message shown here.



Bug Fixes (relative to v4.0 Build 10590 and earlier)

- Attempting to run an acquisition protocol before the ***Plate Heater*** has reached the target temperature will now result in a warning message.
- The ***Flat Field Correction Calibration*** no longer hangs.
- A software error no longer occurs when the static software autofocus range is set below 20um.
- Corrected an issue with the ***2.5D Deconvolution*** imaging mode.
- When using ***Time Series*** mode:
  - Time-lapse scans can now use starting times or time intervals greater than 28800 seconds.
  - For *single-well* and *as-fast-as-possible* time series using *Brightfield* that does not involve ***Liquid Handling*** events, the MOVE_TRANS command will not be used multiple times during a scan.
- Can now set the number of fields when loading a protocol in the case where the field pattern is randomized.
- The Field spacing/Fixed layout setting is no longer always applied.
- The text entry box for the number of fields is no longer limited to 100.
- In the ***Preview*** window:
  - Resolution now changes when you select a different objective.
  - *The Max intensity* value displayed is now correct and no longer causes errors with the *Max intensity* statistic.
- In ***Liquid Handling*** mode:
  - *Aspirate/Dispense* volumes are now limited to increments of 10 µL, rather than 1µL. (The liquid handling mechanism can only operate in increments of 10 µL.)
  - Stage position now resets after incubation.
- When using the *IN Cell Investigator* software for analysis, the Z stacks are now displayed properly in the analysis software, regardless of whether or not the ***Link 3D Parameters*** box has been checked on the ***Protocol Designer:Channel Settings*** card.
- Changing the Windows theme while the software is open no longer influences the correct behavior of the drop-down options from the HWAF and SWAF main toolbar icons or other functionality of the software.

- Wavelengths are now displayed properly in ***Data Review:Explore Image:Choose Wavelengths***, even in cases where a protocol designed with two wavelengths uses only one of those wavelengths in a time series.
- The user interface is now disabled when operating in ***Robotics*** mode. It is no longer possible to close the door or move the plate while the robot is attempting to load or unload plates.
- System performance no longer decreases when:
  - Performing heavy ***3D Deconvolution*** processing
  - Saving large scans involving lots of images to network drives.
- The software can now be uninstalled using the provided uninstall executable file.

Known Issues and Usage Notes

- In ***Review Scan***, ***Object Thresholding*** analysis requires Windows .NET Framework 4.0. On older IN Cell Analyzer systems running .NET Framework prior to v4.0, an upgrade to v4.0 is necessary.

  Workaround: Windows .NET 4.0 can be downloaded and installed from either:
  http://www.microsoft.com/en-us/download/details.aspx?id=17851 (web-based)
  http://www.microsoft.com/en-us/download/details.aspx?id=17718 (stand-alone)
- In ***Data Review*** mode, only one field is available when viewing an image stack.

  Workaround: Reloading the image stack may correct this issue.
- ***3D Deconvolution*** fails if the corresponding OTF file is missing.

  Workaround: Contact GE Technical Support for help locating and installing the appropriate OTF file.
- Pre-existing ***ReviewScan*** protocols that use the *Threshold* measurement must be updated to use the *ImageThreshold* measurement.
- On rare occasions, a plate scan can fail to start due to a threading issue within the IN Cell software. The frequency of this failure is predicted to be roughly 1 in 10000 scans. The actual failure occurs during the previous scan and generates the following report in the IN Cell log file:

  ```
  12/12/2113 15:16:16:544 INFO [Incell Plate Scan] Incell Plate Scan is waiting
  for ICSPollingThread
  ```

  Workaround: When this threading problem occurs, restart the IN Cell system.

  For future software releases, this issue has been fixed as of build 11602.
- When using ***Brightfield*** on an IN Cell 2200, the following Emission filter/Quad combinations should be used:

  Quad 1 / Cy3

  Quad 2 / Texas Red

  Quad 3 / Texas Red

  Note – *When using Quad 2/Texas Red emission, the intensity will be substantially lower than when using Quad / Cy3. Set the Exposure time accordingly to compensate.*
- Network Attached Storage (NAS) performance.

  Data storage on Network Attached Storage (NAS) can be very slow in certain situations. The configurations listed below have been associated with slow performance. There may be other configurations as well.

  1. Java (IN Cell), Windows 7 (workstation), Windows Storage Server 2003 (NAS)
     http://www.sysprobs.com/windows-7-network-slow

2. Java (IN Cell), Windows 7 (workstation), Samba (NAS)
   http://stackoverflow.com/questions/14963927/extremely-slow-file-listing-using-java-samba-win-7

- IN Cell Analyzer startup problems.

  In cases where the IN Cell Analyzer software fails to start, press and hold the "Ctrl" key while launching the program. A console window will appear. The last part of the output will often contain useful information about the failure. For memory allocation failures, follow the instructions in the following item.

- Java Virtual Machine (JVM) memory allocation problem during startup.

  If the IN Cell software fails to start due to a memory allocation problem, use the following steps to reduce the amount of memory requested during initialization:

  1. Open the program configuration file in a text editor:

     "C:\Program Files\GE Healthcare\IN Cell Analyzer 2200\IN Cell Analyzer 2200.lax"

     If necessary, substitute the appropriate scanner model number (e.g. "2000" or "6000").
  2. Modify the "heap size max" setting as shown here. Try changing the value to "1000M" (1 Gigabyte). If that doesn't work, try a smaller value like "800M".

```
#   LAX.NL.JAVA.OPTION.JAVA.HEAP.SIZE.MAX
#   ------------------------------------
#   -Xmx max heap memory setting
lax.nl.java.option.java.heap.size.max=1000M
```

  The software is designed to operate on standard IN Cell workstations. Computers with less memory (like laptops) will often require this modification.

- Installation problem 1 with ReviewScan Analysis files.

  During installation, the ReviewScan analysis software is copied to the normal ANP folder (typically, "C:\ProgramData\GE Healthcare\IN Cell Analyzer 2200\ANP").

  Sites that use Investigator for batch processing also use the ANP folder, which means that certain installations will want to configure a network drive to store analysis protocols. On such systems, the "XANP" setting in the preferences file is manually edited to connect with the site's XANP files on the Investigator workstation. As a result, the INCell acquisition program cannot find the ReviewScan analysis, because the files are installed in the wrong location.

  To manually correct this problem, copy the ReviewScan analysis files from the local ANP folder to the location specified within the following .ini file:

  "C:\ProgramData\GE Healthcare\IN Cell Analyzer 2200\IN Cell Analyzer 2200 Preferences.ini"

  Note that this fix will only work if the Investigator program's XANP folder is available at runtime.

- Installation problem 2 with ReviewScan Analysis files.

  On certain workstations, installation of the ReviewScan analysis software fails. The explanation for this failure is currently unknown, but it appears that local security procedures may be preventing DLLs, EXEs, and ZIP files from being installed to the "ANP" folder. There are three symptoms of this condition:

  1. There are no analyses available in the ReviewScan setup page within the GUI.

2. The installation procedure slows down when copying the ReviewScan files.
3. The ANP folder (typically, "C:\ProgramData\GE Healthcare\IN Cell Analyzer 2200\ANP") is empty.

Contact GE support for help fixing this installation problem.

PAA Overlord - Issues and Workarounds

There are a number of issues that can affect the reliability of automated plate scans using Overlord and IN Cell. Certain conditions can be recovered by pressing the "Retry" button on the Overlord main screen. Many of the problems can be traced to the "Network Attached Storage" performance issues described above.

Information about Overlord and Driver versions:

Testing has confirmed that the following versions of PAA software work well with the IN Cell Analyzer program.

Overlord Main Program: 3.0.23.18

Overlord INCell Driver: 1.0.3.12

For best compatibility between the PAA and IN Cell software, use a driver version greater than 1.0.3.10.

Overlord IN Cell Driver Update Procedure (typical):

1. Shutdown Overlord.
2. Copy the new driver (a DLL file) to C:\program files (x86)\paa\overlord3\commands.
3. From the Overlord commands directory, run the following command:
   C:\> "..\setup\register dlls.exe" Overlord.Commands.GEINcell2000.dll"
4. Restart Overlord.
5. On the ***Setup:Command Setup*** page:
   a) Remove the old version of the command driver (e.g., 1.0.3.3).
   b) Add the new version (e.g., 1.0.3.11). Answer "yes" if asked to restore.
6. Open GEIncell 2000 device. Select Type=2000;, reset the host and timeout if needed
7. Open affected .ovp files. Overlord will remove the old INCell device, because the version has changed. Re-add the new version of the device and then reset the Run.

- Overlord error message: "A script engine for the specified language can not be created." This error message has been traced to corrupted registry setting that can be fixed by following the instructions located at this web address: http://www.mediamonkey.com/support/index.php?_m=knowledgebase&_a=viewarticle&kbarticleid=150

- Network interruptions from other programs on the communication port can cause the Overlord/IN Cell communication to fail.
  To recover from such interruptions, stop the outside program from using the port and then press "Retry" on the Overlord main screen. If necessary, change to a port number other than 9999 using the following setting in the IN Cell main configuration file:

```
<!-- Automation server socket -->
```

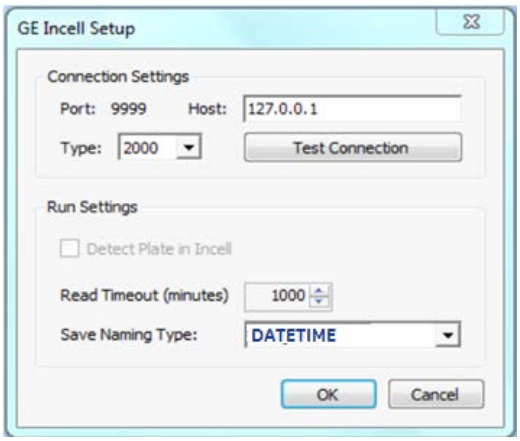
```
<automation_server_socket host="localhost" port="9999" />
```

A similar configuration change will be required for Overlord.

- Slow communication with Network Attached Storage (NAS) systems can cause communication timeouts between Overlord and IN Cell. The chances of this occurring will increase as the number of files and folders in the destination folder on the NAS increases. To reduce the likelihood of failure, apply as many of the following workarounds as possible:
  - Upgrade to driver version 1.0.3.11, or higher.
  - Increase the necessary driver timeout value(s) in " Overlord.Main.exe.config" (only possible if using 1.0.3.11 or higher). Times are in milliseconds. The ClientSendTimeout and ClientReceiveTimeout are the most effective.

```
<?xml version="1.0" encoding="utf-8" ?>
<configuration>
    <configSections>
        <sectionGroup name="applicationSettings"
type="System.Configuration.ApplicationSettingsGroup, System, Version=2.0.0.0,
Culture=neutral, PublicKeyToken=b77a5c561934e089" >
            <section name="Overlord.Commands.GEIncell2000.Properties.Settings"
type="System.Configuration.ClientSettingsSection, System, Version=2.0.0.0,
Culture=neutral, PublicKeyToken=b77a5c561934e089" requirePermission="false" />
        </sectionGroup>
    </configSections>
    <applicationSettings>
        <Overlord.Commands.GEIncell2000.Properties.Settings>
            <setting name="LoadDelayTimeout" serializeAs="String">
                <value>15</value>
            </setting>
            <setting name="GetImagerStateNumberTimeout" serializeAs="String">
                <value>5</value>
            </setting>
            <setting name="IsPlateLoadedTimeout" serializeAs="String">
                <value>5</value>
            </setting>
            <setting name="PreRunCheckTimeout" serializeAs="String">
                <value>1</value>
            </setting>
            <setting name="ClientLingerState" serializeAs="String">
                <value>True</value>
            </setting>
            <setting name="ClientSendTimeout" serializeAs="String">
                <value>60000</value>
            </setting>
            <setting name="ClientReceiveTimeout" serializeAs="String">
                <value>60000</value>
            </setting>
        </Overlord.Commands.GEIncell2000.Properties.Settings>
    </applicationSettings>
</configuration>
```

- Set the "Save Naming Type" to "DATETIME" rather than "UNIQUE".
  Generating unique folder names can be very time consuming (on the order of 10-60 seconds) when working with certain types of remote file storage, e.g., "Network Attached Storage (NAS) performance").



- Disable Java memory clean up procedures by adding "-XX:+DisableExplicitGC" to the "additional" arguments in the INCell .lax file
  In the case of the IN Cell 2000, it may be necessary to add the entire line.

  i. Open the program configuration file in a text editor:

  "C:\Program Files\GE Healthcare\IN Cell Analyzer 2200\IN Cell Analyzer 2200.lax"

  Substitute the appropriate scanner model number (e.g. "2000" or "6000").

  ii. Look for the following line and add the setting shown in italics.

  # LAX.NL.JAVA.OPTION.ADDITIONAL

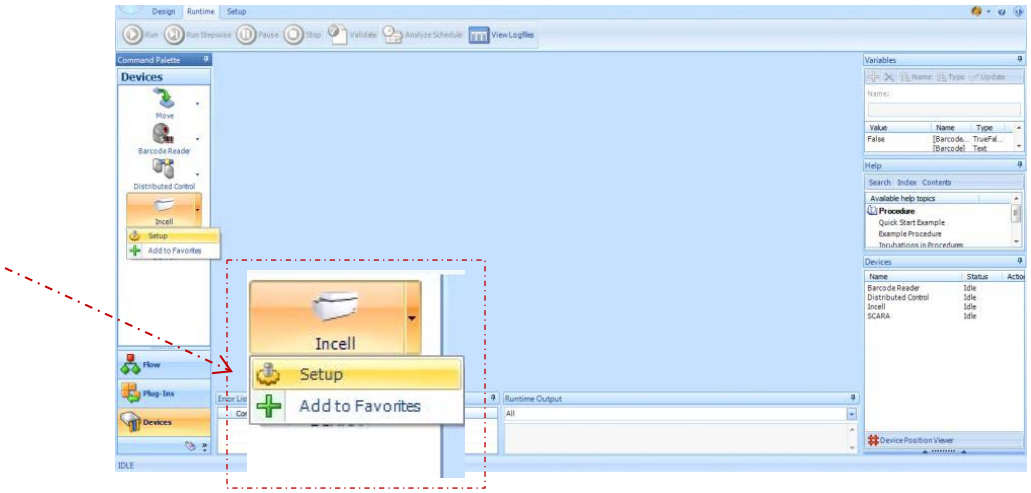
  # ----------

  lax.nl.java.option.additional=-XX:+UseConcMarkSweepGC ***-XX:+DisableExplicitGC***

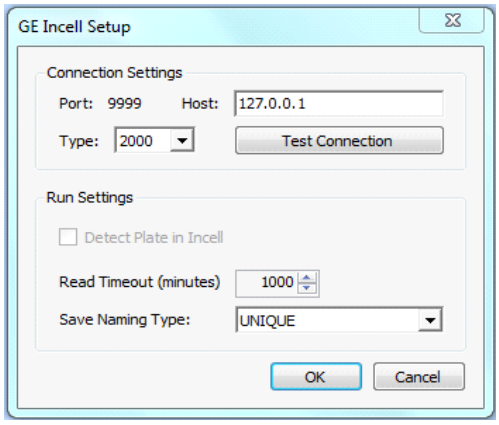
- Overlord timeout during long scans (driver version < 1.0.3.10).

  Scans that last longer than the Overlord "Read Timeout" value may result in a communication failure between Overlord and IN Cell. To workaround this problem, increase the device communication ***Read Timeout*** value to a value that is larger than the time required to scan a plate. Using a large timeout such as 1000 minutes will prevent a communication problem between the IN Cell acquisition and Overlord software.

  1. Open the ***Overlord*** software by clicking the desktop icon or navigating to *C:\Users\incell\AppData\Local\Programs\PAA\Overlord3\Overlord.Main.exe*. The ***Overlord*** console opens.
  2. Under ***Devices***, right-click ***Incell*** and select ***Setup*** from the pop-up menu.



3. On the ***GE Incell Setup*** dialog, increase the ***Read Timeout*** interval to *1000* minutes. Click ***OK*** to save and close the dialog.



- The Overlord IN Cell driver has a limited buffer size for accepting protocol lists returned from the remote control message <m:ImagerStatus>. Prior to version 1.0.3.12, the limit was 8Kb. Starting with version 1.0.3.12, the limit was increased to 64Kb. To work-around this limit, reduce the number of acquisition protocol files (.xaqp files) stored in the AQP folder and then restart the IN Cell software. In the case of the 2200, the AQP folder path is "C:\ProgramData\GE Healthcare\IN Cell Analyzer 2200\AQP".
- Acquisition protocol file names that contain unusual characters like "&" will cause communication problems between IN Cell and Overlord. The symptom of this problem is a parsing error reported in the Overlord log file. Error messages will use terminology like "unrecognized token". The parsing failure will also cause Overlord to disconnect from the TCPIP socket. The IN Cell client will then report that the remote control client has disconnected. To avoid this issue, use .xaqp file names with simple alphanumeric characters. Do not use characters like "&" or "%".

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