

# **IN Cell Analyzer**

## **Acquisition Software, Version 6.0**

### **Release Notes**

## Introduction

Version 6.0 of the IN Cell Analyzer software contains improvements, optimizations, and fixes over version 5.2.

Upgrading from previous versions (like 4.6, 5.0, 5.1, or 5.2) should require only minimal training. The new features and interface changes should be intuitive for most users.

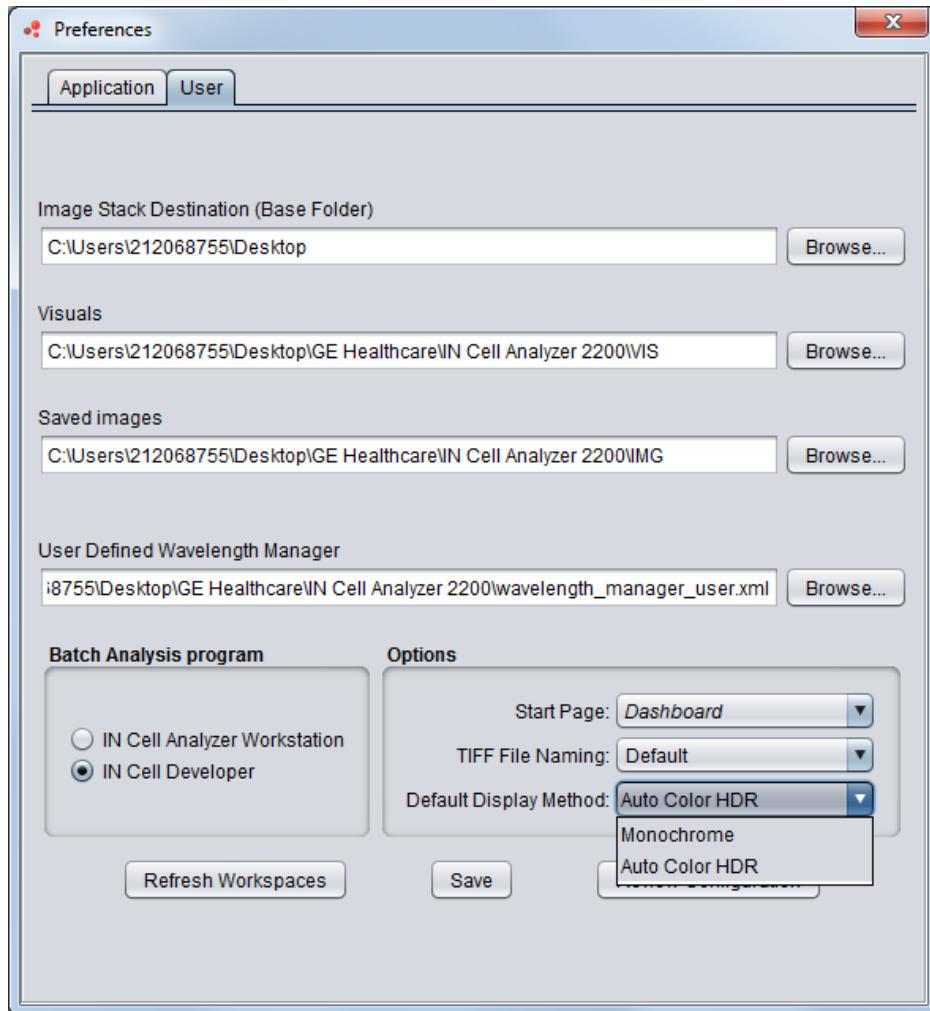
The following release notes describe the primary changes between versions 5.2 and 6.0 of the IN Cell Analyzer software. Additional information can be found in the release notes from previous versions of software, which are included within the 6.0 installers.

## New Features

### Automatic Display Color Switching - High Dynamic Range Color Mapping (1564)

Starting with V6.0, the default display method will be "Auto Color HDR". Images will automatically be displayed with a color that matches the current emission filter's wavelength. In addition, the color look-up-table will transition to grayscale at the top end of the dynamic range.

The default display method is applied to all display windows, including those used in the *Dashboard*, *DataAcquisition*, and *DataReview*. Previous versions of software used "Monochrome" as the default display method. For sites that prefer a different default display method (for example, "Monochrome"), set the "Default Display Method" in the preferences dialog, as shown in the following screenshot.

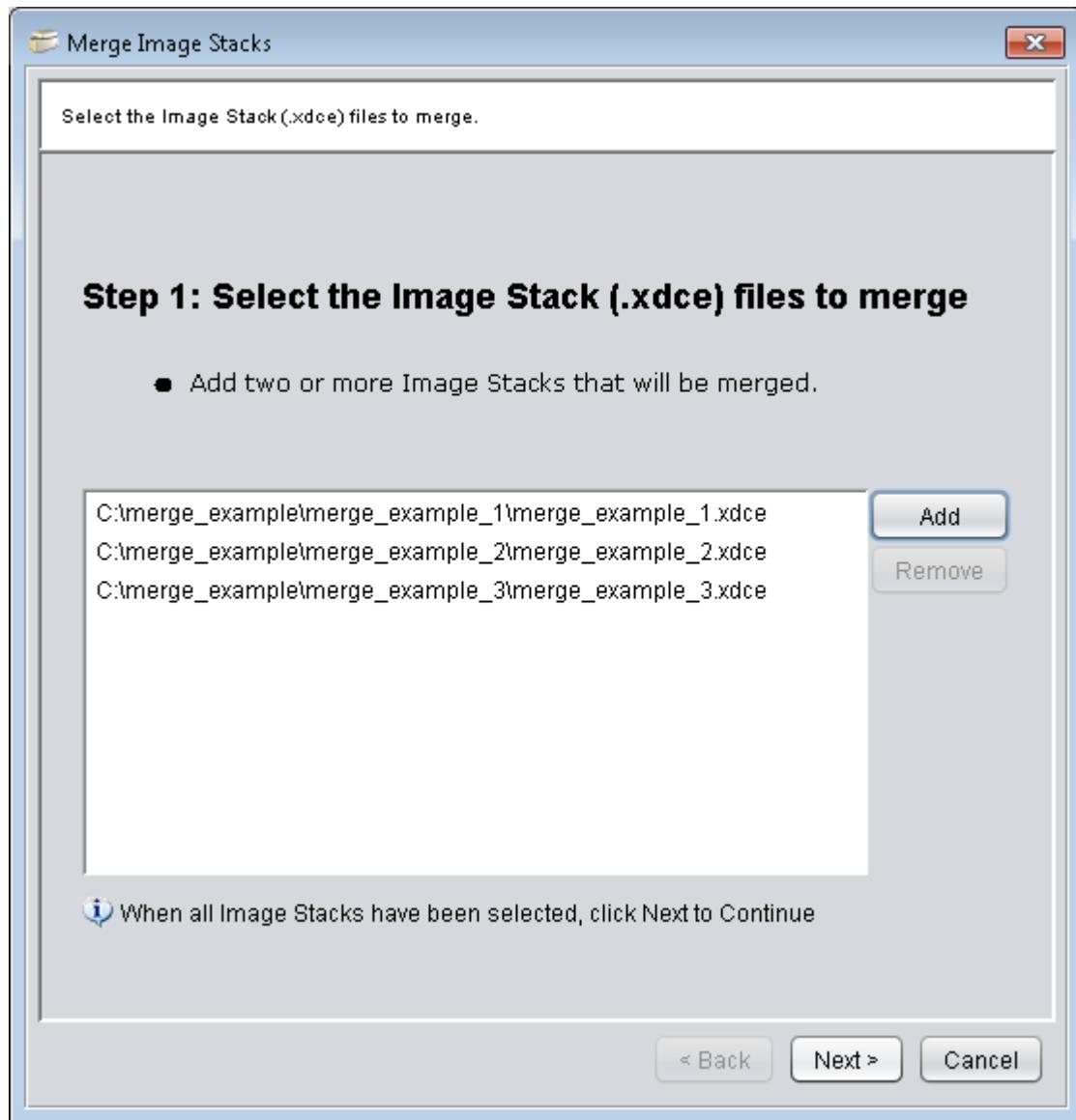


**Color Display Improvements (1633)**

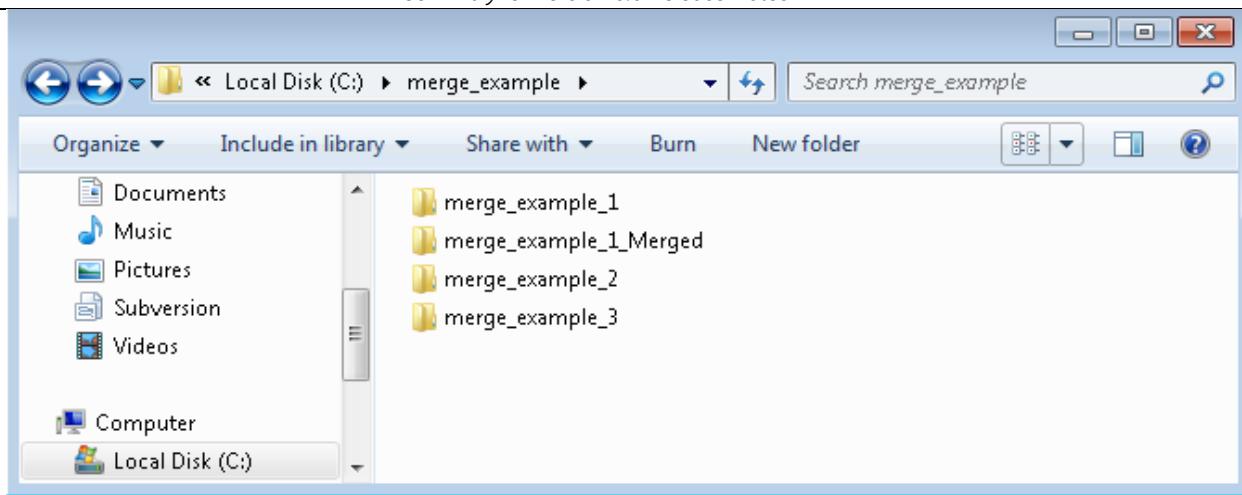
In addition to the high dynamic range color maps, two other changes were made to the normal color maps. First, an Orange color has been added for use with probes like Cy3 and TexasRed. Second, the blue, green, and red color maps have been improved to display more detail.

**XDCE File Merge Tool (821)**

Merging XDCE files after scanning enables a variety of new screening experiments. The plate can be removed for incubation, pipetting, modification, etc. between scans. Merged XDCE files provide a method of consolidating scans into a single, multi-timepoint experiment.



Merge Involving Three Time-Lapse Scans



#### Important notes about the merge tool:

- XDCE files must be acquired with identical (or very similar) acquisition protocols. For example, the number of channels, channel ordering, fields, and Z sections must be the same.
- Merged files will be stored in a new folder. TIFF images will be copied from the original folders. No files will be deleted.
- Incompatible XDCE files will not be merged.
- Merged XDCE files cannot be merged again with other XDCE files. It is only possible to merge files a single time. To add additional XDCE files, repeat the entire merge using the original XDCE files.

GE recommends testing the merged results before use with critical experiments. Not all XDCE files can be merged; it is important to know whether the acquisition protocol generates results that can be merged. In general, the same acquisition protocol should be used for all runs that need to be merged.

Note that XDCE files generated by previous versions of software are potentially merge-able, depending on the conditions.

#### **Improved Plate Heating Status Reporting for the Lid & Bottom Heaters (1455, 1640, 1654)**

The plate heater dialog will now present the complete status of both heaters (bottom and lid). When the current temperature reaches the target temperature, the color highlight will change from red to green. When heating elements are enabled, the instrument will estimate the necessary set points needed to reach the desired target. The column titled "Set Point (C)" shows the temperature that the instrument is trying to reach. The column titled "Current (C)" shows the measured temperature of the heater indicated by the column on the left.

**Plate Heaters**

Target Plate Temperature:  °C

	Set Point (°C)	Current (°C)	On Time (hh:mm:ss)
Bottom Heater	39.0	35.2	00:00:02
Lid Heater	35.0	—	00:00:00

Both Heaters Off  
 Bottom Heater On / Lid Heater Off  
 Both Heaters On

**Below Temperature**

**Plate Heaters**

Target Plate Temperature:  °C

	Set Point (°C)	Current (°C)	On Time (hh:mm:ss)
Bottom Heater	39.0	39.0	00:01:15
Lid Heater	35.0	—	00:00:00

Both Heaters Off  
 Bottom Heater On / Lid Heater Off  
 Both Heaters On

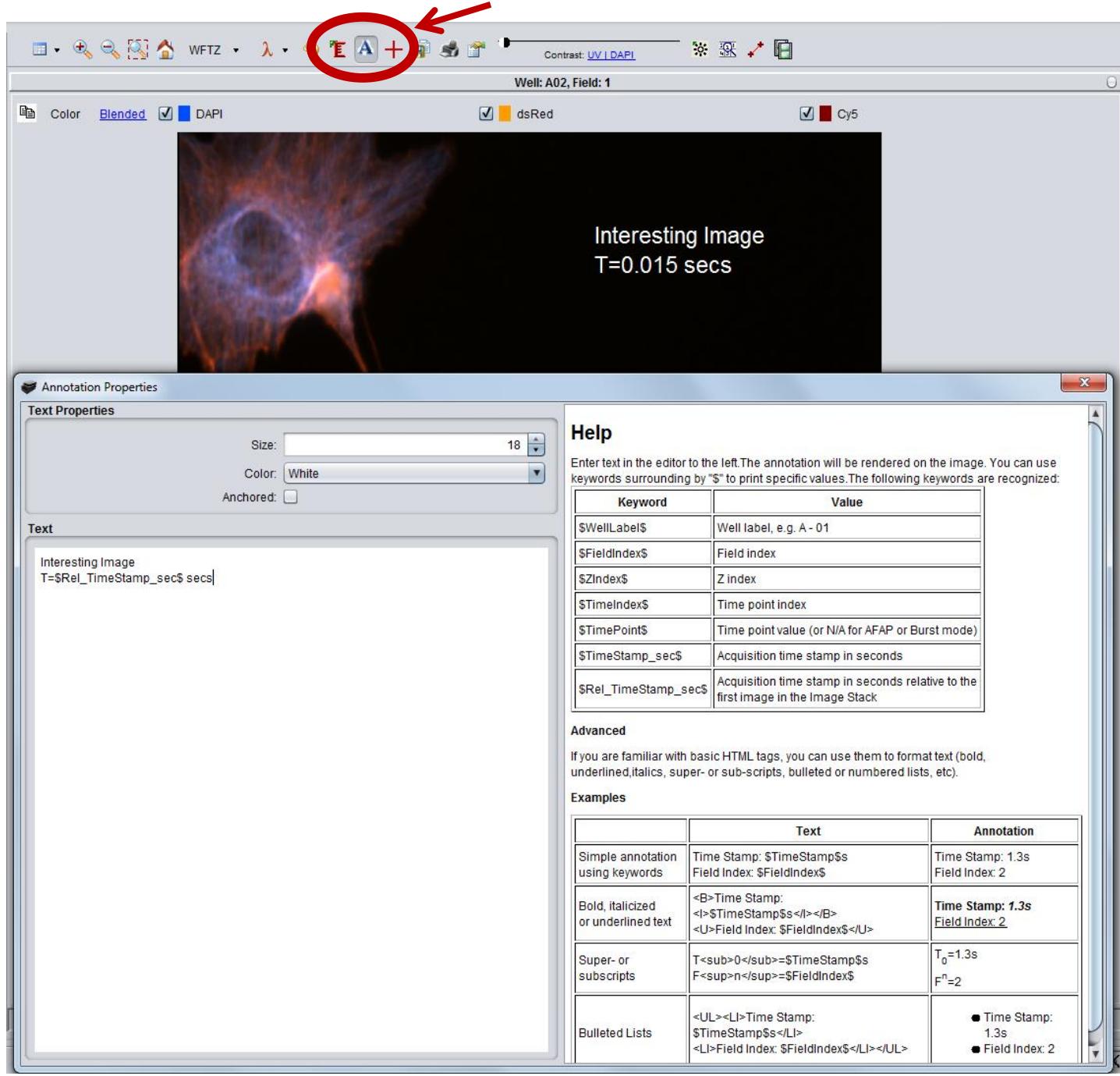
**At Temperature**

IN Cell uses a simple thermal model that estimates the relationship between the requested plate temperature and the instrument's set point. The actual temperature of the plate (especially within the wells) is unknown. Empirical testing may be required in order to determine the optimum temperature settings. The IN Cell heating element and associated circuitry have been shown to have stable performance. The thermal properties of the plate, however, will have a large effect on the final temperature within the wells.

For best results, allow the heaters and the plate to equilibrate for at least one hour before scanning. Because all plates are different, there is no perfect method of estimating the actual temperature of the sample.

### Annotation for Images Displayed in DataReview and AVI Movies (1304)

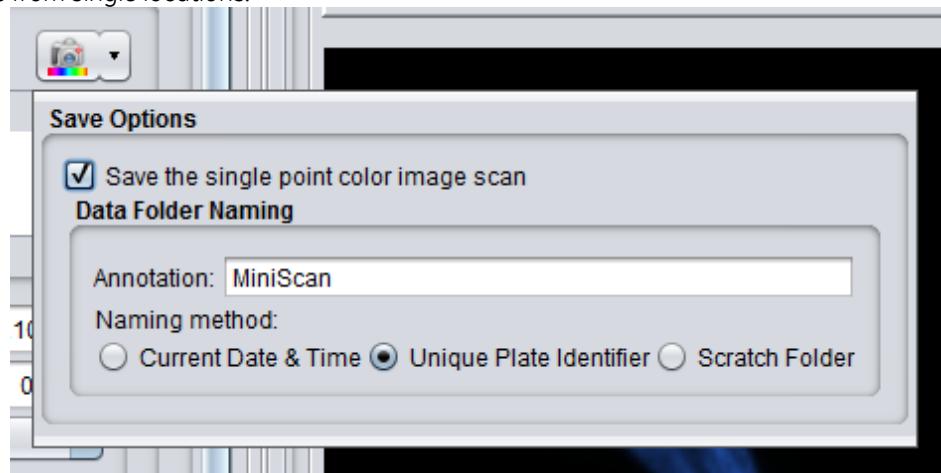
A variety of annotations can be added to the image display panel during *DataReview*. To add annotations, modify the "Text" section of the *AnnotationProperties* page, which can be displayed by pressing the icon labelled "A" at the top of the image display. Information visible within the *DataReview* image display will also be visible in AVI movies.



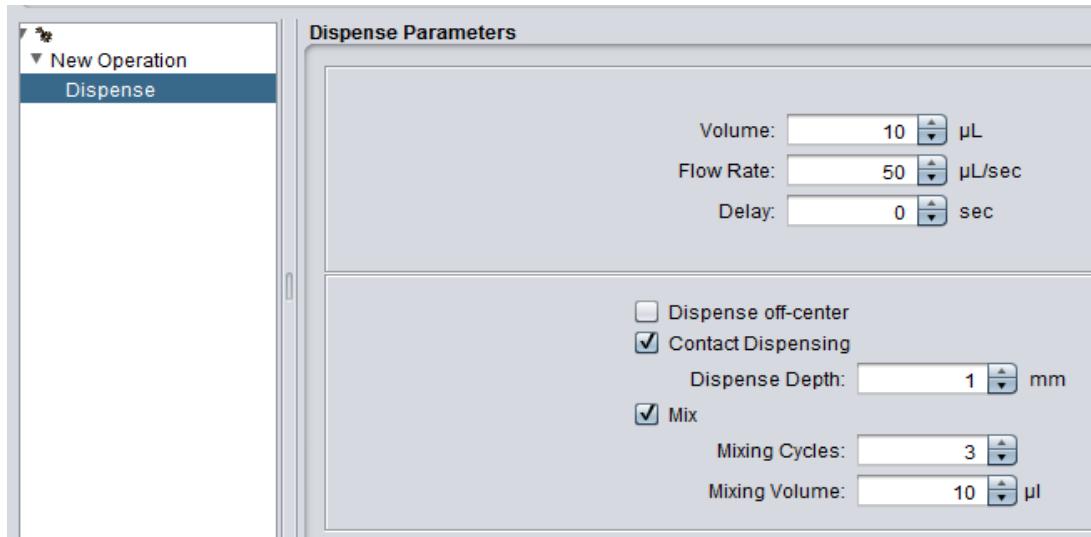
Annotation Tool

**Interactive Mode Image Recording (1595)**

The MiniScan button now has an option (see pull down arrow) for accessing options to quickly save images without a full scan. By default, MiniScan does not save images. Enable "Save the single point color image scan" feature for interactively acquiring and recording images from single locations.

**Liquid Handling Mixing Option (1358)**

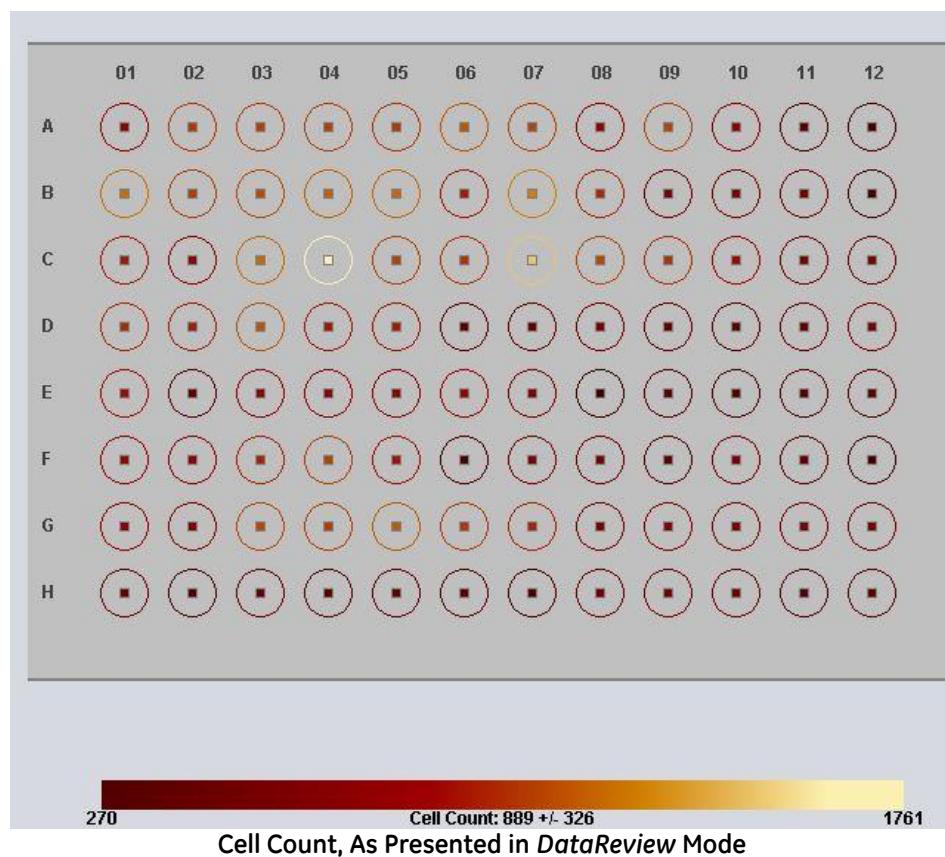
Liquid dispensing can now be combined with a mixing procedure that consists of aspiration and dispense cycles, as shown in the screenshot below. To use mixing, contact dispensing must also be enabled and the dispense depth must be greater than zero.



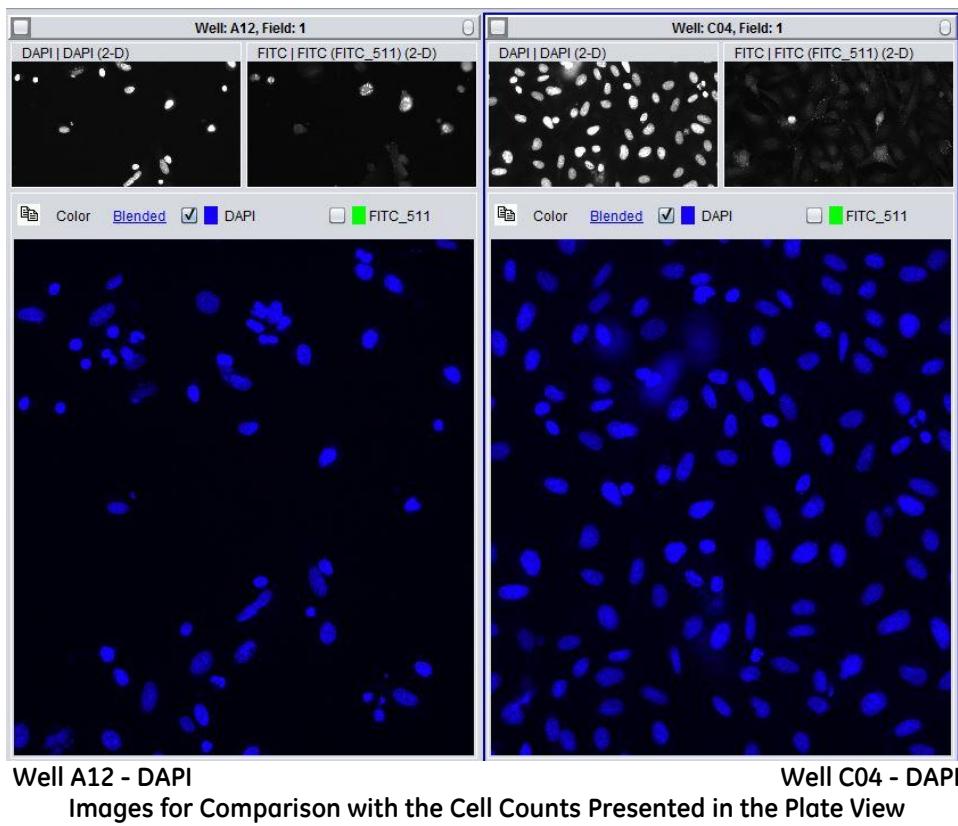
## General Improvements

### Cell Counting (1242)

After a scan with cell counting enabled, the number of counted objects can now be viewed in the *DataReview*'s plate map and also in the XDCE file.



Cell Count, As Presented in *DataReview* Mode



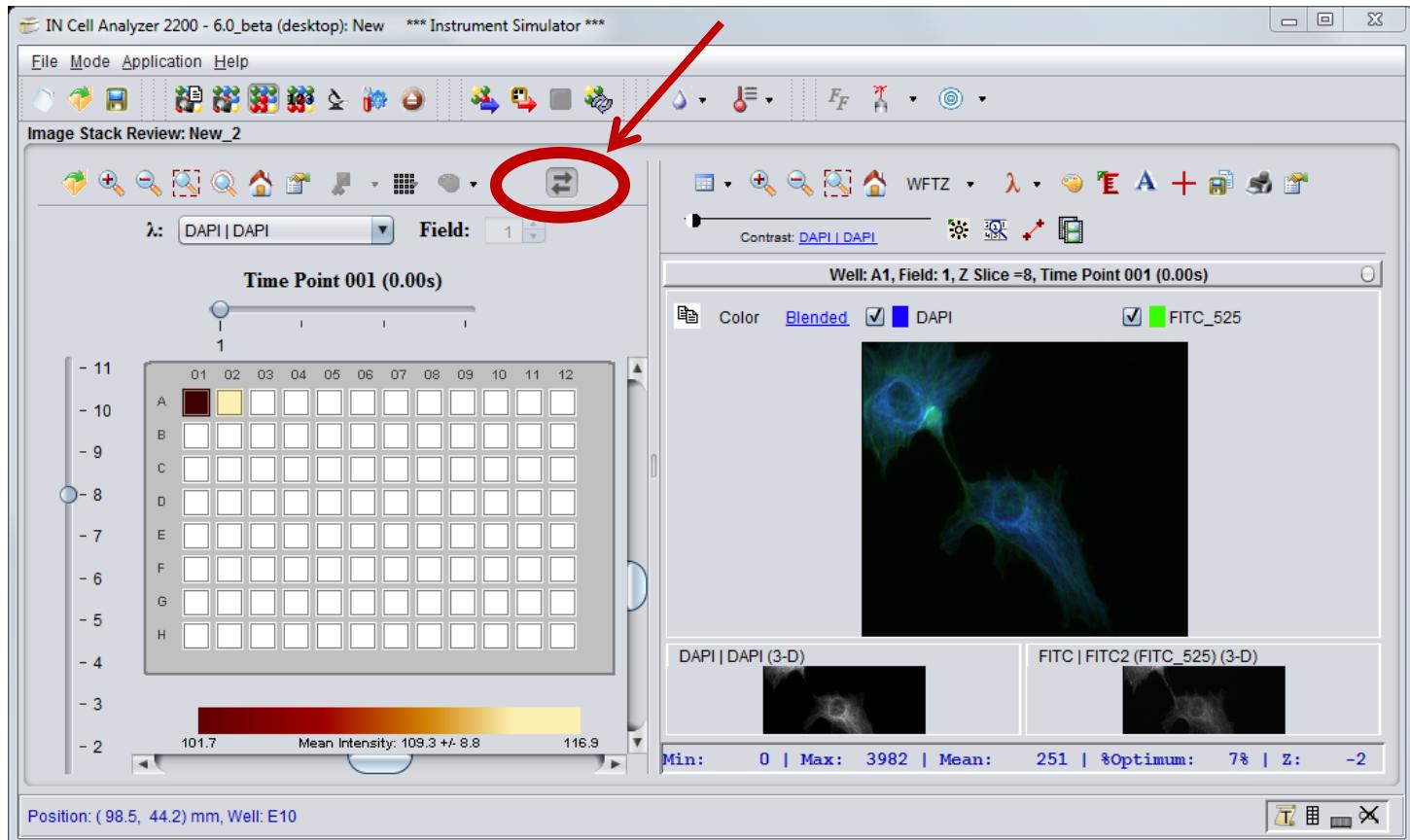
Images for Comparison with the Cell Counts Presented in the Plate View

**Time-stamp in the DataReview Heatmap (1380)**

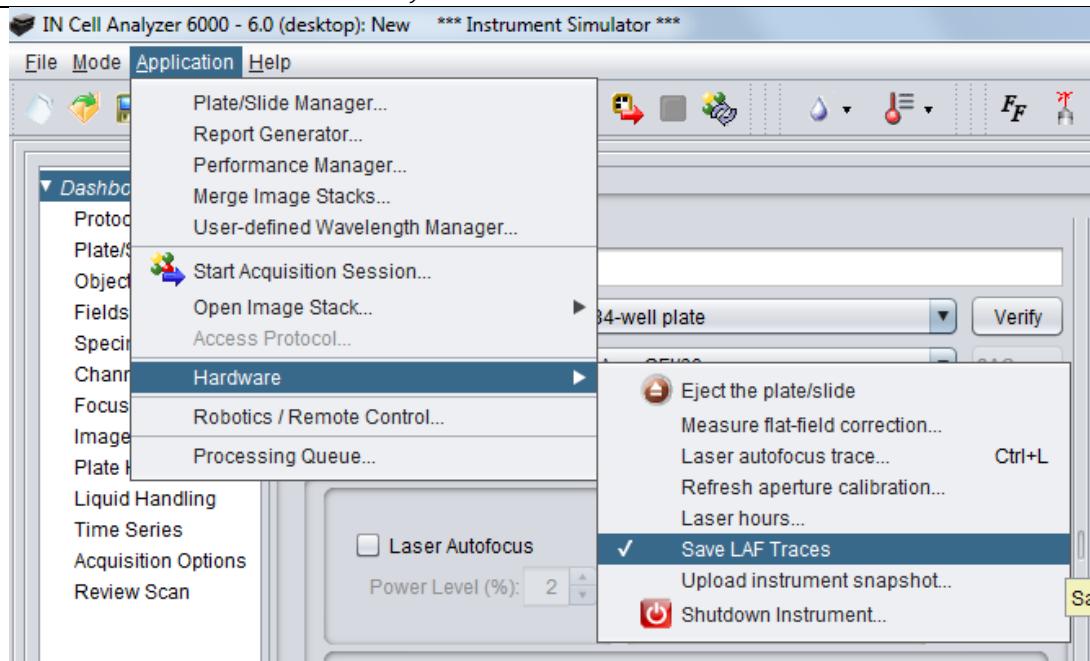
The image acquisition time can now be viewed in the DataReview's plate map, similar to the situation with other scan results like mean intensity, focus position, and cell count.

**Z-Slice and Time-point Sliders in DataReview Heatmap (1424)**

The Z and T sliders in the DataReview heatmap can now be connected with the image display. To connect the DataReview and the image view, press the button highlighted in the following figure. When enabled, changing the Z or T index of the heatmap will update both the heatmap and the image display. Similarly, updating the WFTZ image selection will update the heatmap.

**New Option for Saving LAF Traces During Scanning (1614)**

To help diagnose LAF issues that occur during scanning, enable the option shown below and then rerun the scan. CSV files containing LAF traces will be recorded next to every TIFF image that used laser autofocus. After the scan, use the DataReview tool to identify which well(s) had focus problems and then find the corresponding CSV file(s). In many cases, the information recorded in the CSV file will provide valuable clues about the cause of failure. For example, the 2nd LAF peak may be missing due to an optical blemish on the surface of the plate. Alternatively, the LAF scan range may have missed the desired surface of the plate. Additional clues can often be found in the CSV files from adjacent wells.



### Remote Control Dialog Has A New Panel for Displaying Messages and Errors (1621)

The RemoteControl dialog box contains a new panel for reporting messages and errors. In the example below, INCell is reporting that the remote client has requested a non-existent or invalid acquisition protocol.

**Protocol name:** RC test

**Base folder:** C:\

**Review Scan folder:**

**Image stack:** C:\RC test\RC test h 2015.10.08.07.58.22

**Annotation:** h

**Status and Communication**

Date / Time	Received	Sent	State
2015.10.08 07:59:02.812		ImagerState (5)	
2015.10.08 07:59:02.811	GetImagerState		
2015.10.08 07:59:02.299		ImagerMessage : Start to scan new well B11	
2015.10.08 07:59:01.808		ImagerState (5)	
2015.10.08 07:59:01.808	GetImagerState		
2015.10.08 07:59:00.806		ImagerState (5)	
2015.10.08 07:59:00.806	GetImagerState		
2015.10.08 07:58:59.809		ImagerMessage : Start to scan new well B12	

**Messages and Errors**

Date / Time	Type	Message
2015.10.08 07:58:00.398	ERROR	Unable to open protocol requested by remote client RC_testxaqp.
2015.10.08 07:53:36.266	ERROR	Unable to open protocol requested by remote client RC_testxaqp.

### Nominal Plate Surfaces Displayed in the FocusFinder (1597)

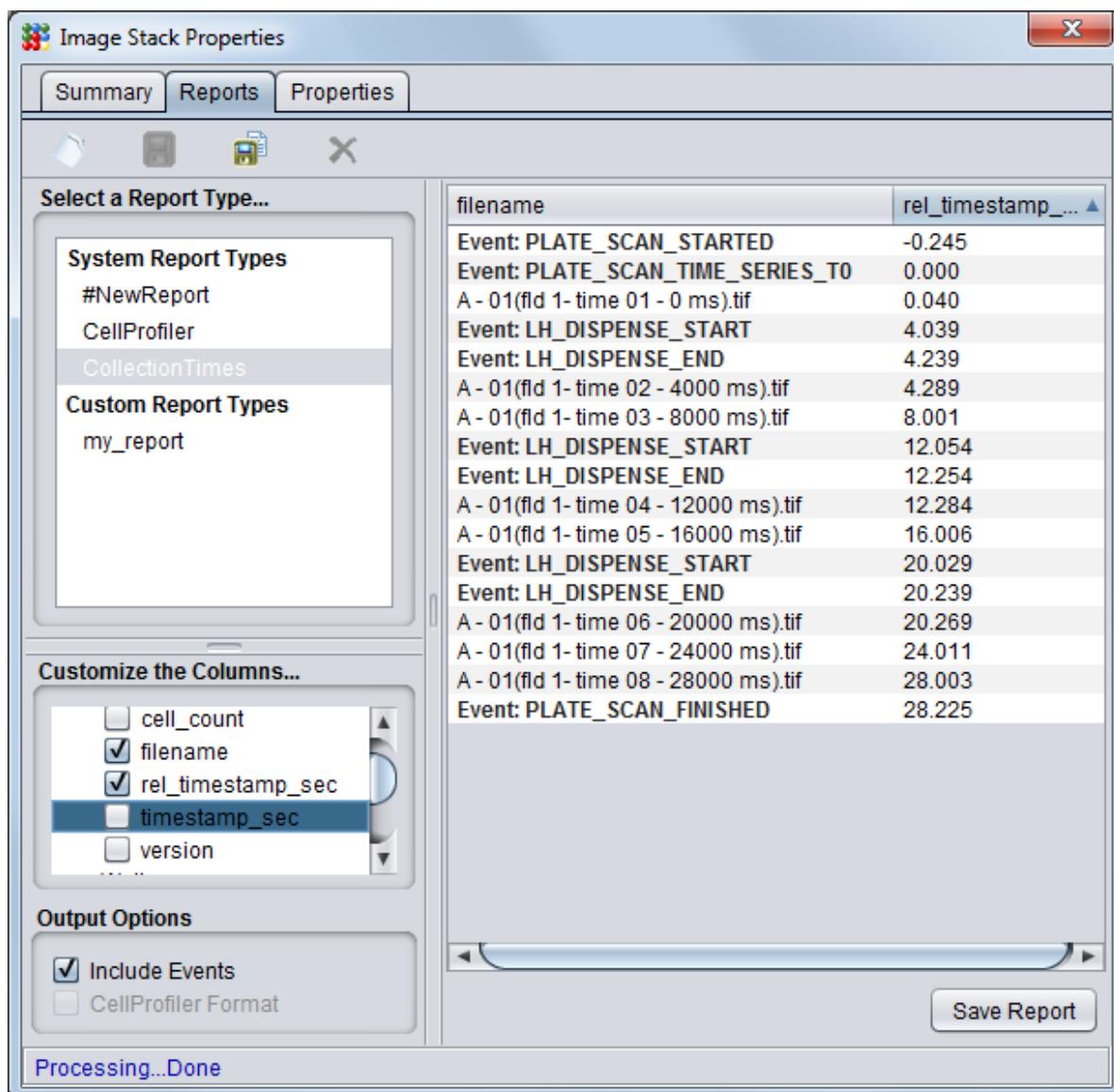
The expected locations of the plate bottom and nominal focus are now represented on top of the FocusFinder graph. A red triangle is used for the bottom height and a green triangle is used for the nominal focus. The graphics can be used in two ways:

first, to confirm that the configuration settings match the actual plate; and second, to help find the correct focal plane during interactive imaging.



### Time-stamps for Liquid Handling Dispense (1596)

Time-stamps are now recorded at the start and end of dispense operations. Similar to the image time-stamps, the absolute time values are recorded in the XDCE file in "Epoch time" format. The *ReportGenerator* can be used to view the relative time-stamps, as shown in the following figure. Enable the "Include Events" toggle to view the time-stamps.



## Discontinued Development for IN Cell Analyzer 2000

GE has discontinued development of the IN Cell Analyzer software for 2000 class instruments. Sites that want to upgrade their 2000 should use V5.2 instead of V6.0.

## Known Issues and Usage Notes

Information about previously reported topics can be found in previous versions of the release notes, which are located in "C:\Program Files\GE Healthcare\IN Cell Analyzer XX00\manual". Only new or updated items are described here.

## Significant Fixes

Version 6.0 contains mainly new features, small enhancements, and small bug fixes. None of the items fixed in V6.0 qualify as "severe". For a full list of changes and fixes, see the table in the next section.

Items 1618, 1626, 1628, 1654, and 1667 are probably the most significant fixes, although it is unknown whether any sites were actually affected by these issues. All of these items were identified by GE during internal testing. The issues existed in previous versions of software and none of them were reported by users.

## List of Changes Between 5.2-14311 and 6.0-14420

Items listed in this table have been fixed in version 6.0, unless otherwise discussed in the comments.

<u>ID</u>	<u>Brief Description</u>	<u>Additional Comments</u>
821	Create a tool for merging similar/identical XDCE files.	The ability to merge XDCE files makes it possible to remove plates/slides between time-points.
1242	Add number of counted objects to the list of available <i>DataReview</i> heatmaps.	The number of counted objects can be useful when reviewing the results of a plate scan.
1304	Provide annotation capabilities for adding time stamps in AVI movies.	See release notes for additional information.
1358	Add mixing capability to the LH dispense event (within the well)	See release notes for additional information.
1380	Add relative timestamps to the list of available features in the <i>DataReview</i> heatmap	Timestamps can be useful when comparing images of living cells from wells and fields.
1424	Z-slice and Timepoint sliders should also update the displayed image.	The Z and T sliders in <i>DataReview</i> mode will now update the heat map and the displayed images.
1431	RPM installer should avoid showing std IP address if initial connection is successful	Only affects the installation GUI. Does not affect the function of the RPM installer.
1433	Rapid time-series for single well liquid handling.	The time between the end of the dispense and the start of the first image is less than 0.5 seconds. Also see #1638 and #1596.
1455	Improved status reporting of the plate heaters (top & bottom)	The new dialog box provides information about the actual state of the instrument.
1485	Unneeded warning messages when enabling/disabling encoders on channels that don't have encoders (e.g. liquid handling motors)	Warning messages like the following were recorded in the instrument controller log file. The messages were not helpful, because they referred to a benign condition. In this case, the LHZ motor does not even have an encoder.  40 33.047 fullstep.cpp:384 enableEncoder:> Failed to disable encoder on LHZ 40 33.056 fullstep.cpp:375 setEncCountsPerRev:> Failed to set encoder counts on LHZ to 0
1494	Stopping a scan confuses the graphical layout in <i>DataReview</i> .	The problem was actually benign. Only the graphical appearance was affected.
1525	GUI controls for ASAC adjustment needs improvement.	Some of the controls behaved strangely when double-clicked.
1526	Changing the plate type after LAF verification should generate a warning message.	Done.
1561	Add the image XY coordinates to the XDCE file	The XY coordinates for each image are saved in client coordinates. XY coordinates are useful for ThirdParty software and possibly for future <i>ReviewScan</i> features.
1564	Color enhanced LUTs with auto switching to EM channel	The default image display now uses a color LUT that corresponds to the wavelength. To change the default, set the appropriate user preference.
1572	LUTs cannot be set while in microscope mode	Fixed.
1575	Duplicate channels can't be viewed in <i>DataReview</i> .	<i>DataReview</i> mode was confused by certain types of channel replicates.
1593	Instrument homing procedure in xyz.py doesn't always switch to the 10X objective lens.	The homing procedure did not switch to the 10X objective lens when used with a 2200 or a 6000. The workaround was to position the 10X before beginning the procedure.
1595	Simplify the methods for recording images at the current location (interactive mode)	See the release notes for information about new MiniScan features.
1596	Provide a method of reviewing LH and image time-stamps together.	LH time stamps are now recorded at the start and end of dispense operations. Use the <i>ReportGenerator</i> to view the time-stamps.

1597	Display the plate bottom height & nominal focus in the <i>FocusFinder</i>	Viewing the nominal plate surfaces is helpful in two ways: 1) quick verification of the plate settings; and 2) finding focus.
1598	Add a "Delete All" time-points method	Use the right mouse button to delete all time-points. The new method is easier than the pre-existing method, which involved selecting all of the time-points and then pressing delete.
1600	Add support for SCIVAX plates	Plate types have been added for 24, 96, and 1536 well SCIVAX plates.
1610	Exposure time problem when centering on objects after <i>FocusFinder</i>	The exposure time is now properly reset between use of the <i>FocusFinder</i> and the object centering tools.
1612	PackardView 96-well plate type contains inaccurate bottom thickness	The nominal plate bottom thickness has been changed to match the manufacturer's specifications. The changes will only take effect on new installations, however, because the installers do not overwrite pre-existing XPLT files.
1613	GUI should handle the case where the ICS doesn't find enough <i>LAF</i> peaks.	A warning is now presented.
1614	Provide the "Save <i>LAF</i> Traces" option for normal operation.	Use this feature to save <i>LAF</i> traces (as CSV files) for diagnostic purposes.
1617	GUI should report the condition where the Image AF saturates.	A warning message will be displayed if the image AF saturates.
1618	<i>ReviewScan</i> fails if first scan has no excluded fields.	In the case where the first scan did not exclude any fields, subsequent <i>ReviewScan</i> protocols would fail. Fixed.
1619	Unnecessarily verbose logging when transferring log files from the instrument	The upload status was being written to the log file while recording log files from the scanner. The resulting log files were potentially a lot larger than necessary.
1621	INCell should present a visible warning if Remote Control client requests an invalid acquisition protocol	In the case where a remote control client requested an invalid (or non-existent) acquisition protocol, INCell quietly used the current protocol as a fallback. The new warning message should help avoid future problems and confusion.
1622	Add calculated "Optical Bottom Thickness" to the plate information table.	The optical bottom thickness is calculated from the actual bottom thickness divided by the index of refraction of the imaging substrate.
1623	Update customer support phone numbers for systems located in France.	The phone number changed.
1624	Add a method of determining the laser enabled hours on the 6000.	A simple table with the laser hours can be displayed by selecting "Applications" -> "Hardware" -> "Laser hours..." from the main menu.
1626	Default Z step size for the 20X/0.75 (id 12207 and 12208) is too coarse.	The default Z step size is one of the parameters needed for image based AF. The coarse value may have caused imprecise focus results on the 20X/0.75. To check the active settings, look at both the site specific and the factory default GUI configuration files.
1627	"No Z Slices" message displayed by the GUI no longer makes sense.	The message was being displayed whenever the 3D imaging was not enabled. Users did not need this information, and it caused confusion.
1628	Error when opening a single channel, timelapse protocol in the case where a cell counting protocol is already open.	The error was caused by a mismatch of the acquisition protocol settings.
1629	Update Java VM from 8u20 to 8u51	A routine update to get Java fixes and enhancements. No known issues were addressed by this upgrade.
1630	Make the default color map for the image viewers a user preference.	See #1564.
1632	Protocol marked as modified when it shouldn't have been.	Fixing this will help reduce the number of times people get prompted to save "modified" acquisition protocols.
1633	Add an "Orange" color map, and adjust the "Blue", "Red", and "Green" color maps	Orange is useful for Cy3. The red, green, and blue color maps are brighter and more usable.

1634	Update and record the required instrument control software versions.	Minimum versions required by V6.0: ICS 23798 IO Board 2200 1.0020 (same as V5.2) IO Board 6000 1.0023 (same as V5.2) Nano3 1.0028 (same as V5.2)
1635	Allow sites to adjust the min & max allowed LH dispense rates	The default min and max values have not been changed. Sites that need slower or faster flow rates can modify the limits by editing the GUI configuration file. The pump is calibrated at 90 ul/sec.  <liquid_handling_minimum_flow_rate>50</liquid_handling_minimum_flow_rate>  <liquid_handling_maximum_flow_rate>200</liquid_handling_maximum_flow_rate>
1638	Optimize LH pump motor control.	The commands used to control the LH pump motor have been optimized. Redundant setup commands have been eliminated.
1640	Plate/lid heater controls are confusing.	The heater control toggles were confusing. See #1455 for related information.
1646	Update the Operator's Manual to get Russian translations	Not actually done in V5.2. The V5.2 Release Notes incorrectly stated that this item was already completed. The V6.0 installers contain the Russian translation.
1654	Handle case(s) where the lid heater is disconnected.	When the lid heater was disconnected, the AD conversion electronics reported a temperature of "47.1 C". Rather than reporting an invalid temperature, the GUI will now report that the lid heater is disconnected, and the operator will only be allowed to enable the bottom heater (not the lid heater).
1663	Pointlist image display jumps laterally in <i>DataReview</i> mode.	Fixed.
1666	Inconsistent TIFF image file naming when using 3D and 2.5D deconvolution.	The deconvolution procedure used a slightly different method of generating TIFF image file names. In certain situations, the deconvolution's method generated different names than the method used for all of the other acquisition methods. Deconvolved image files may have followed a naming different pattern, relative to the other acquisition methods. Starting with V6.0, all acquisition methods will use the same file naming procedure. This discrepancy has existed since the original version(s) of the INCell software.  ThirdParty software that relies on file naming patterns should be easier to develop. Likewise, file sorting methods will generate the expected sequence of TIFF files. IN Cell has not been affected by this problem, because the exact file names are retrieved from XDCE files rather than from directory listings.
1667	Prevent XY stage moves before LH dispense operations are complete. Retract needle before moving laterally.	Certain LH dispense sequences may have resulted in XY motion (e.g. to the next well) before the LH needle was fully retracted from the well. The problem only occurred in the case where the needle was deep into the well and an image was not acquired after the dispense.
1671	<i>DataReview</i> 's plate view window background turns black under certain conditions.	The plate view background sometimes turned black under the following conditions: - enable thumbnail display - enable "Display All Fields" - adjust Z section slider for 3D channel - select the 2D channels
1678	Update the Liquid Handling control tooltips.	Additional information about the purpose of the buttons in the LH control dialog box would be helpful.

1696	Add IO board firmware v1.0023 to the 2200 installation packages	Version 1.0023 may be needed to fix USB communication issues that happen on newer IO boards.  As of 17-Nov-2015, version 1.0020 is still the official version for the 2200.
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