NextSeq v4.0 Software Release Notes

NextSeq Control Software v4.0.0

NextSeq Service Software v4.0.0

NextSeq System Suite Installer v4.0.0

Local Run Manager v2.2.1

Real-Time Analysis v2.11.3

Universal Copy Service v1.5.6

NextSeq Recipe Fragments v4.0.0

DMA Driver v4.5.3

For NextSeq 500/550 and NextSeq 550Dx (Research Mode)

Introduction

These Release Notes detail new features, improvements, and issue fixes for the NextSeq 500/550 Sequencing System and the NextSeq 550Dx Sequencing System (Research Mode). This software release is only compatible with Windows 10 operating system and is not backwards compatible with Windows 7.

For more information on the NextSeq 500, NextSeq 550, or NextSeq 550Dx, see the following guides available on www.illumina.com

- NextSeq 500 System Guide (document # 15046563)
- NextSeq 550 System Guide (document # 15069765)
- NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)
- NextSeq 550Dx Research Mode Instrument Reference Guide (document # 1000000041922)

The software package includes:

- NextSeq Control Software v4.0.0
- NextSeq Service Software v4.0.0
- NextSeq Software System Suite v4.0.0
- Local Run Manager v2.2.1
- Real-Time Analysis v2.11.3
- Universal Copy Service v1.5.6
- NextSeq Recipe Fragments v4.0.0
- DMA Driver v4.5.3
- Windows 10 Operating System support
- Scanning functionality for Infinium MethylationEPIC BeadChips

I. NextSeq Control Software v4.0.0 (NCS)

NEW FEATURES:

- Added ability to scan the Infinium MethylationEPIC BeadChip on NextSeq 550 instruments.
- Incorporated Local Run Manager software
    - Uploading projects to BaseSpace Sequence Hub requires you to set up runs in Local Run Manager rather than in BaseSpace Prep tab. Data generated are equivalent.
- Removed support for BaseSpace Onsite, BaseSpace Prep Tab, and BaseSpace Broker
- Changed Operating System to Windows 10
- NCS v4.0 is not backwards compatible with Windows 7
- NCS v4.0 includes support for NextSeq v2.5 flow cells on NextSeq 500/550 instruments and on the Research Mode only of NextSeqDx instruments.

- Windows Explorer is now enabled while running NCS.
- NCS v4.0 now runs as an application instead of a custom user interface or kiosk mode.
- NextSeq instruments running NCS v4.0 operate using Windows 10 OS which is designed to access samba/CIFS shares on SMBv2 protocol by default. NextSeq instruments running NCS versions prior to v4.0 and using a SMB server or NAS, must upgrade to the SMBv2 protocol upon upgrade.
- Installation of all Illumina software moved to C:\Program Files
- All files previously installed on C:\ have been moved to C:\Program Data
- Logs that were previously on C:\ have moved to D:\Illumina\[application]\Logs
- Updated the run setup workflow:
- Run mode setup is now setup using Local Run Manager Mode or Manual Mode and can be configured with run monitoring or run monitoring and storage on BaseSpace Sequence Hub.
- NCS v4.0 requires run output folder to be at least two levels up from a network location, for example:
    - Network\folder1\folder2, where folder2 is the output folder.
- Added support for workgroups in BaseSpace Sequence Hub
- Simplified selection of custom recipes in Manual Mode.
- View Terms updated for Instrument Performance Data
- Improved Run Review screen
- User now has ability to edit run parameters before proceeding to the sequencing screen
- Consumables are not validated against the configured run when they are loaded using the manual run creation workflow in NCS v4.0
- Updated instrument maintenance prompts.

GENERAL NOTES

- Flow cell registration takes longer than expected during the first run after the NCS v4.0 upgrade. This is expected behavior due to internal firmware downloading from instrument components.
- In Windows 10, Services console is used to reconfigure user accounts. Use Local Run Manager to change user account configuration to run Local Run Manager components under a *network* (not local) account.
- For CytoSNP customers:
    - NCS v4.0 generated gtc files are compatible with GenomeStudio 2.0 and BlueFuse Multi v4.5.
    - NCS v4.0 generated gtc files are not compatible with previous GenomeStudio versions (i.e. 2011.1 or 2010)
    - User must select "gtc and idat" for the Output File Type.
- For Methylation customers:
    - NCS v4.0 does not generate gtc files.

- GenomeStudio 2.0 is not designed to analyze Methylation data.
- Users must install GenomeStudio Software 2011.1 to analyze Methylation data from idat files.

IMPROVEMENTS:

- The SequencingComplete file is now created before the start of purge/wash.
- BeadChip scan now copies SDF file to output folder.
- Enable Auto-Start after successful pre-run checks is set by default.
- Run setup 'Next' button is always enabled. If 'Next' is clicked and any field is invalid, a dialog box will appear explaining the first incorrect field, so the user can correct it.
- Updated the auto center algorithm used during BeadChip scanning to be more robust.

ISSUE FIXES:

- Fixed an intermittent issue where a NullReferenceException would occur when entering the Load Buffer screen.
- Fixed an issue where the wash state was updated to Wash Complete when an error was encountered in the wash preventing its completion.
- Fixed an issue where the software was showing the instrument as connected to the network even when the network cable was disconnected.
- Corrected BeadChip completion time.
- Fixed an issue where an old run folder in the temp directory was not being deleted.
- Fixed an issue that prevented the Network Configuration Setting from being saved.
- Updated Autocall for BeadChip scanning to v4.0.

NEXTSEQDX RESEARCH MODE UPDATES AND FIXES:

- Includes support for NextSeq v2.5 flow cells on the Research Mode of NextSeqDx instruments.
- RTA 2.11.3 is the default RTA version for Research Mode of NextSeqDx instruments, whereas Diagnostic Mode uses RTA 2.9.5.
- System prompts user to remove RUO consumables remaining on instrument when user selects shutdown or reboot to Diagnostic Mode of NextSeqDx instruments.
- When using Research Mode of a NextSeqDx instrument, the option was added to allow the user to reboot back into Research Mode. Previously, any restart would boot into Diagnostic Mode.
- Research Mode will prompt user to confirm the instrument wash status when instrument is rebooted from Diagnostic Mode.
- Fixed an issue where the Operating System would reboot to Diagnostic Mode after flashing firmware on the Research Mode. Now, the Operating System reboots to Research Mode.

KNOWN ISSUES:

- Software does not log the completed cycles in the Run Parameters file.
- The Pre-Run Checks output file (PreRunChecks.csv) does not contain all tests run.
- All tests are included in the User Interface screen and contained in instrument log files.
- The Flow Cell door does not close on the Run setup Screen. Must return to Home screen
- The BaseSpace Run Mode is not displayed on the Run Review Screen.
- During Pre-Run checks, only a warning message is displayed if there is no connection to BaseSpace Sequence Hub, the user is not given option to proceed without Basespace.
- Software does not give the user the option to proceed without BaseSpace during the sequencing run if the BaseSpace connection fails.
- A failed BaseSpace connection does not use consumables or cancel run in BaseSpace. The run ends without piercing consumables and user is notified of the error.
- If the user enters index cycles greater than the number of read cycles the system validates properly and prevents workflow progression, but no warning message is provided to the user.
- User is not allowed to proceed with invalid run parameters.
- Incorrect base pair yield value is displayed in the Q Score graph during a sequencing run. However, the correct Estimated Yield (Gb) value is displayed in the summary table to the right of the graph.
- The run output folder path is incorrectly assigned and requires user to edit the Local Run Manager-reported output folder path to assign the correct output folder path.
- The instrument's Wash State does not persist in NCS v4.0 after a restart or shutdown. NCS will ask the user if they would like to wash the instrument upon starting up after a restart or shutdown.
- A sample sheet is incorrectly indicated as required for runs using BaseSpace Run Monitoring Only, as denoted by an asterisk next to the field title.
- Data transfer to external output location is stalled if user is locked out due to incorrect login credentials. Data transfer will resume upon successful login. In the interim data is stored locally. This situation only impacts data transfer and not run analysis.
- Local Run Manager rejects Dx v2.5 flow cells at loading when run on NextSeqDx Research Mode. Use Manual Mode instead of Local Run Manager Mode to run Dx v2.5 flow cells on Dx Research Mode.
- The run progress bar on the sequencing screen has limited accuracy during read 1 of the first few runs after upgrading software to NCS v4.0.
- The IndexMetricOut.bin file exists in the Run Folder on the instrument, but it is not transferred to the Output Folder. Users who wish to maintain this file must manually transfer the file before run is deleted.

- The Temp folder can be deleted prior to completely uploading to an output folder when runs are older than 7 days or there are more than 3 Local Run Manager runs contained in the temp folder.

II. NextSeq Service Software 4.0.0 (NSS)

NEW FEATURES:

- Updated Autocall for BeadChip scanning to v4.0
- Enabled Service Software application to run when the logged in user (to Windows) is a non-admin.
- Changed installation locations to allow for non-admin use
- Created Windows Event Log source in NSS v4.0.
- Updated WashState.xml to "unknown" after fluidics test or pump control.
- Updated copyrights and splash screens in NCS, NSS, NSSI
- First Time Setup and Preventive Maintenance results are now produced in PDF format.
- All tests now check that the appropriate consumables are loaded.
- Implemented Illumina Proactive in Service Software.
- Added support for the NextSeq v2.5 kit.
- Updated the auto center algorithm used during BeadChip scanning to be more robust when there are problems in the auto center location of the BeadChip.

ISSUE FIXES:

- Fixed an issue where the System Status Icon of an error (Red X) would not disappear if there was also a RAID warning displayed (Orange Wrench).
- Fixed an issue where the FC registration results don't show in UI during System Check or Preventive Maintenance.
- Fixed an issue where the software would accept pump volumes greater than the maximum.
- Fixed an issue where the system would display an error when the output folder is set to root of drive.
- Fixed and issue where the System Check flow rate test result and details do not show up on detailed result screen.

III. NextSeq System Suite Installer v4.0.0 (NSSI)

NEW FEATURES:

- Added Universal Copy Service (UCS)
- Updated .NET Framework dependency to v4.5.2
- Removed the BaseSpace Broker
- Moved Recipes to Program Data

- Removed Sequencing Analysis Viewer (SAV)
- Removed the End User License Agreement
- Updated Recipe Fragment Version to v4.0.0

IV. Local Run Manager v2.2.1

NEW FEATURES:

- Add ability to download or upload HTTPS site certificate
- Improved logic in parsing reference genome files used for secondary analysis
- Added support for disk space check on network directories
- Minor UI enhancements and fixes
- Update Local Run Manager guided tutorial to reflect UI updates

KNOWN ISSUES:

- Disk space check for secondary analysis is not performed when importing a run
- On rare occurrences, the final folder name in the Output Run Folder path can get appended an extra time to the Output Run Folder path that is displayed on the Run Overview page
- Custom Primer selection in the UI does not get updated in Local Run Manager.
- Read Type and Custom Primers defined in the Software will not be reflected in Local Run Manager.
- Custom Primers selected in the Software are not updated in Local Run Manager.
- The GenerateFASTQRunStatistics.xml file that is created at the end of the GenerateFastQ workflow returns 0 for each statistic.

V. Real-Time Analysis v2.11.3 (RTA)

NEW FEATURES:

- Updated low diversity detection in template generation
- Improved bubble detection
- Changed install location
- Now allows software to run with a non-admin user logged into Windows
- Updated InterOp DLLs from v1.1.0 to v.1.4.1.
- Still maintains backwards I/O compatibility with older versions
- Bug and Performance fixes
- The addition of ImageMetrics, which displays minimum and maximum contrast on raw pixels
- The addition of ExtendedTileMetrics, which displays occupancy information in BaseSpace (if RTA is appropriately configured)

- new revisions of CorrectedIntMetrics (deprecating: C1intensity, SNR, CorrectedIntensities) and QMetrics (compressing binned Q-scores better)

ISSUE FIXES:

- Fix normalization bug – for index read starting with G
- Merge index logic to maintain the intensity ratio between 2 channels during index intensity normalization to accommodate red being much brighter than green, or vice versa
- Switched from using 0.1% Phi X per tile as threshold on measuring and reporting Phi X error rate to absolute cluster count of 2500 per tile. This was done to improve the accuracy of the reported Phi X error percentage when the spike-in is very low as the previous method would lead to severe quantization of the per tile reported value.

VI. Universal Copy Service v1.5.6 (UCS)

NEW FEATURES:

- Replaces Run Copy Service and BaseSpace Broker with Universal Copy Service.
- The system will warn the user if Universal Copy Service is actively uploading to BaseSpace during a shutdown attempt.

KNOWN ISSUES:

- A slow internet connection while uploading a large file may cause UCS to hang until network speed improves.