

NextSeq v4.0.1 Software Release Notes

NextSeq System Suite Installer v4.0.1

NextSeq Control Software v4.0.1

NextSeq Service Software v4.0.1

Local Run Manager v2.4.0

Real-Time Analysis v2.11.3

Universal Copy Service v1.6.1

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 the v4.0.1 software release 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. Features no longer supported in NextSeq v4.x software are BaseSpace Prep Tab and BaseSpace Onsite.

- For more information on previous releases, visit the Illumina Support Site. NextSeq v4.0.0 Software Release Notes (document # 1000000070338)

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 Software System Suite v4.0.1
- NextSeq Control Software v4.0.1
- NextSeq Service Software v4.0.1
- Local Run Manager v2.4.0
- Real-Time Analysis v2.11.3
- Universal Copy Service v1.6.1
- NextSeq Recipe Fragments v4.0.0
- DMA Driver v4.5.3

I. NextSeq System Suite Installer (NSSI)

NEW FEATURES v4.0.1:

- Updated NCS and NSS from version 4.0.0 to 4.0.1
- Update Local Run Manager from version 2.2.1 to 2.4.0

II. NextSeq Control Software (NCS)

NEW FEATURES v4.0.1:

- Added fan usage statistics to Instrument Performance Data.
- Enhanced security by communicating with Local Run Manager through HTTPS.

ISSUE FIXES v4.0.1:

- Improved accuracy of progress bar on sequencing screen to be more proportional to cycles elapsed.
- Fixed an issue where Local Run Manager was unable to setup impersonation and excessively timing out.
- Addressed a rare firmware timing issue that caused Bit Error Rate (BER) pre-run check test failures
- File deletion service made more robust.
- Corrected output folder path by removing the redundant Run Id in the folder name.
- Fixed an issue where wash state would not always persist. Now wash state will maintain its current state on NextSeq 500/550 RUO instruments.
- Removed an asterisk on the Run Setup screen, indicating that a Sample Sheet is required, even when it was not. An asterisk is displayed next to the Sample Sheet input box only if using BaseSpace storage or VeriSeq.
- Corrected yield displayed, yield now displays Mb/Gb/Tb on Sequencing screen.
- Fixed an issue where the Temp folder could 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.
- The following issue has been fixed within individual Local Run Manager modules. See Local Run Manager module release notes for more details - The IndexMetricOut.bin file located in the Run Folder on the instrument, was not transferred to the Output Folder. Users who wished to maintain the file needed to manually transfer the file before run was deleted.

NEXTSEQDX RESEARCH MODE UPDATES AND FIXES:

- Fixed issue that rejected Dx v2.5 flow cells and reagents on NextSeqDx Research Mode when using Local Run Manager. Now, Dx v2.5 flow cells and reagents are accepted on NextSeqDx Research Mode.

KNOWN ISSUES v4.0.1:

- 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 copying data between 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.
- Consumables are not validated against the configured run when they are loaded using the manual run creation workflow in NCSv4.0
- For CytoSNP customers:
 - NCSv4.0 generated gtc files are compatible with GenomeStudio 2.0 and BlueFuse Multi v4.5.
 - NCSv4.0 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:
 - NCSv4.0 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.
- 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, 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.
- User is not shown a red X on the Pre-Run Checks screen when there is no internet connection during a BaseSpace run. Only a warning message is shown. Run will timeout after pre-run checks but before piercing consumables.
- After the NCS v4.0.0 to NCS v4.0.1 upgrade, the prior version Local Run Manager v2.2.1 will appear in Windows Programs and Features although it is actually not installed. The new version Local Run Manager v2.4.0 will be installed and run as intended. To resolve this issue, Local Run Manager v2.2.1 can be manually removed.
- Copyright year is 2018 on Control Software Splash and About Screens
- If the NextSeq is off-domain, and storage is on-domain, Local Run Manager will not be able to access the stored data for on-board analysis. To resolve this issue, it is recommended that:
 - Local Run Manager Off-Instrument is used (e.g. installed on the computer on the same domain)
 - The NextSeq and Storage match (e.g. both on-domain or both off-domain)
 - Transfer, via Windows File Explorer, the data to the NextSeq for local analysis

III. NextSeq Service Software (NSS)

NEW FEATURES v4.0.1:

- Enhanced security by communicating with Local Run Manager through HTTPS only.

ISSUE FIXES v4.0.1:

- No new issue fixes

IV. Local Run Manager

NEW FEATURES v2.4.0 (NSSI v4.0.1):

- Integrated UCS v1.6
- Added functionality to enable sending Local Run Manager analytics logging to BaseSpace. When an analysis completes and if Instrument Performance Data is turned on, a serialized analysis info object is written to the analysis folder of that analysis. The log content includes various metrics including installed modules, install custom prep kits and various statistics about the completed analysis. This log file is uploaded to BaseSpace using UCS after it is written.

ISSUE FIXES v2.4.0 (NSSI v4.0.1):

- No new issue fixes

KNOWN ISSUES v2.4.0 (NSSI v4.0.1):

- 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 User Interface 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.
- The GenerateFASTQRunStatistics.xml file that is created at the end of the GenerateFastQ workflow returns 0 for each statistic.

V. Real-Time Analysis (RTA)

VERSION 2.11.3

- No changes

GENERAL NOTE:

- 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 (UCS)

NEW FEATURES v1.6.0 (NSSI v4.0.1):

- Improved data integrity for BaseSpace upload.
- Improved data security for BaseSpace uploads.
- UCS waits for all required folders before marking a run as “complete”.

ISSUE FIXES v1.6.0 (NSSI v4.0.1):

- Large file uploads no longer fail data integrity check
- Errors in upload contract validation now have consistent and informative error messaging.

KNOWN ISSUES v1.6.0 (NSSI v4.0.1):

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