

# **NextSeq 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 v1.6.0**

**NextSeq Recipe Fragments v4.0.0**

**DMA Driver v4.5.3**

**.NET Framework v4.6.2**

**For NextSeq 500/550 and NextSeqDx (Research Mode)**

**September 2019**

## 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).

For more information on the NextSeq 500, NextSeq 550, or NextSeq 550Dx, see the following guides available on [www.illumina.com](http://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 v1.6.0
- NextSeq Recipe Fragments v4.0.0
- DMA Driver v4.5.3
- .NET Framework v4.6.2
- Windows 10 Operating System (OS)
- Scanning functionality for Infinium MethylationEPIC BeadChips

## I. NextSeq System Suite Installer (NSSI) v4.0

### NEW FEATURES v4.0.0:

- Added Local Run Manager
- Updated Real-Time Analysis (RTA) to v2.11.3
- Added Universal Copy Service (UCS)
- Updated .NET Framework 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

## II. NextSeq Control Software v4.0 (NCSv4.0)

### NEW FEATURES 4.0.0:

- Added ability to scan the Infinium MethylationEPIC BeadChip on NextSeq 550 instruments.
- Incorporated Local Run Manager Software
- Changed OS to Windows 10
- NCSv4.0 is not backwards compatible with Windows 7 (NCSv2.2 and NCSv3.1)
- NCSv4.0 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.
- NCSv4.0 now runs as an application instead of a custom user interface or kiosk mode.
- NextSeq instruments running NCSv4.0 operate using Windows 10 OS which is designed to access samba/CIFS shares on SMBv2 protocol by default. For NextSeq instruments running NCS versions prior to v4.0 and using a SAMBA server or NAS, it is recommended to upgrade storage to SMBv2 protocol.
- 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.
- NCSv4.0 requires run output folder to be at least two levels deep within the network UNC path, for example:
  - \\server\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
- Updated instrument maintenance prompts.

### NEW FEATURES 4.0.1:

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

#### 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 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.

#### 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.

#### NCS4.0.0 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.

#### **NCS4.0.1 ISSUE FIXES:**

- 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:**

- Includes support for NextSeq v2.5 flow cells on the Research Mode of NextSeqDx instruments.
- RTA 2.11 is the default RTA version for Research Mode of NextSeqDx instruments, whereas Diagnostic Mode uses RTA 2.9.
- 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.
- 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:**

- 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 NCSv4.0.0 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 4.0 (NSS v4.0)

#### NEW FEATURES 4.0.0:

- 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 (NextSeq Suite Installer)
- First Time Setup and Preventive Maintenance results are now produced in PDF format.
- All tests now check that the appropriate consumables are loaded.
- Implemented Instrument Performance Data.
- 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.

#### NEW FEATURES 4.0.1:

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

#### 4.0.0 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 flow cell 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 an issue where the System Check flow rate test result and details do not show up on detailed result screen.

### IV. Local Run Manager

#### NEW FEATURES v2.2.1 (NSSI v4.0.0):

- Added ability to download or upload HTTPS site certificate.

- Improved searching logic for 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

#### **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:**

- Users now allowed to set Service Accounts to local admin users.
- If user uploads a site certificate, it will now be uploaded to C:\program files\illumina\Local Run Manager\downloads.
- Analysis service runs as 64 bit. All other services run as 32 bit.

#### **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 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 v2.11.3**

#### **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

## VI. Universal Copy Service

**NEW FEATURES v1.5.6 (NSSI v4.0.0):**

- 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.

**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:**

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