



# nCounter<sup>®</sup> Analysis System

# **User Manual**

This manual applies to both the nCounter *MAX* Analysis System and the nCounter Dx Analysis System with *FLEX*.

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Introduction
Product Use Limitations5
Product Components5
Overview of the Procedure5
Manual Conventions
Instrument Specifications6
Environmental Requirements6
Safety Information (English)7
Caution Symbols7
Informations de Sécurité (Français)8
Symboles Attention
Hardware Overview9
General Information9
Prep Station10
Digital Analyzer11
Selecting the Instrument Mode
Selecting the Instrument Mode
Selecting the Instrument Mode
Selecting the Instrument Mode    12      Prep Station    13      Select Instrument Mode    13
Selecting the Instrument Mode       12         Prep Station       13         Select Instrument Mode       13         Switch Instrument Mode       13
Selecting the Instrument Mode       12         Prep Station       13         Select Instrument Mode       13         Switch Instrument Mode       13         Digital Analyzer       14
Selecting the Instrument Mode       12         Prep Station       13         Select Instrument Mode       13         Switch Instrument Mode       13         Digital Analyzer       14         Select Instrument Mode       14
Selecting the Instrument Mode12Prep Station13Select Instrument Mode13Switch Instrument Mode13Digital Analyzer14Select Instrument Mode14Switch Instrument Mode15
Selecting the Instrument Mode12Prep Station13Select Instrument Mode13Switch Instrument Mode13Digital Analyzer14Select Instrument Mode14Switch Instrument Mode15Operating the Prep Station16
Selecting the Instrument Mode12Prep Station13Select Instrument Mode13Switch Instrument Mode13Digital Analyzer14Select Instrument Mode14Switch Instrument Mode14Switch Instrument Mode15Operating the Prep Station16Prior to Initiating a Run16
Selecting the Instrument Mode12Prep Station13Select Instrument Mode13Switch Instrument Mode13Digital Analyzer14Select Instrument Mode14Switch Instrument Mode15Operating the Prep Station16Prior to Initiating a Run16Waste Removal16

Operating the Digital Analyzer
Enterprise Package
User Management
Login Settings
USB/FTP Settings
Archive Settings
Cartridge Tracking Settings
Audit Report Generation
Overview of File Structure
Navigating File/Folder Screens
Reporter Library Files
Upload RLFs via USB Flash Drive35
Upload RLFs via FTP37
Cartridge Definition Files
Creating a CDF on a Personal Computer40
Upload CDFs via USB Flashdrive45
Upload CDFs via FTP46
Creating a CDF on the Digital Analyzer48
Initiating a Run53
Pausing a Run
Completing a Run60
Download RCC files via USB Flashdrive60
Download RCC Files via E-mail61
Download RCC Files via SSH61
Download RCC Files via FTP62
Technical Support & Maintenance65
Technical Support65
Prep Station Maintenance
System Setup67



System Hardware70
System Software
Troubleshoot
Shutdown System
Digital Analyzer Maintenance
System Setup
System Software
Troubleshoot90
User Profile91
System Information91
Disk Cleanup91
Shutdown System93
Cleaning94
Prep Station94
Digital Analyzer94
Disposal of Electronic Equipment94
Symbols and Definitions95



# Introduction

## **Product Use Limitations**

The nCounter Analysis System is intended for applications for which approval/clearance is not required (nCounter Dx Analysis System with *FLEX*) and for research use (nCounter *MAX* Analysis System). Procedures pertaining to the Dx (*FLEX*) version of the system are addressed in a separate manual (MAN-C0034). The nCounter Analysis System may only be used with NanoString's nCounter reagents. See the documentation that accompanies the reagents for information regarding performance and sample type limitations.

# **Product Components**

The nCounter Analysis System consists of two instruments: the Prep Station and the Digital Analyzer. A power cable is included with each instrument. The Prep Station also includes a pipette tip rack, a liquid waste container, a solid waste container, and silicone grease. Customers may expand the system by adding additional Prep Stations.

# **Overview of the Procedure**

- 1. After sample processing and hybridization has been completed according to the assay or reagent instructions, samples are loaded onto the Prep Station, where they are purified and immobilized onto the internal surface of a sample cartridge. This process takes 2-3 hours, depending on the number of samples run and the sensitivity setting selected.
- 2. The sample cartridge is then transferred to the Digital Analyzer for imaging and analysis.
- 3. During processing with the nCounter Analysis System, e-mail alerts may be used to track sample status if the system has been networked and properly configured (optional).
- 4. Finally, results are available for download directly from the Digital Analyzer.



# **Manual Conventions**

The following conventions are used throughout this manual and are described for your reference.

Text Style	Meaning
	Bold text is typically used to highlight a specific button, keystroke, or menu option. Bold text may appear elsewhere to highlight important text or terms.
Italic	Italic text is typically used to highlight references to another section of the manual. Italic text may also be used to highlight references to other manuals or instructional material. These are often accompanied by a hotlink that will take you directly to the referenced document or section.

**NOTE:** This notation indicates general information that may be useful for improving assay performance. These notes may clarify other instructions or provide guidance to improve the efficiency of the assay workflow.

**CAUTION:** This notation indicates the potential for bodily injury or damage to the instrument if the instructions are not followed correctly. Always carefully read and follow the instructions accompanied by this symbol to avoid potential hazards.

**IMPORTANT:** This notation indicates important information that is critical to ensure a successful assay. Following these instructions may help improve the quality of your data.

### **Instrument Specifications**

Test Lanes per Run	1-12
Weight	Prep Station 5s: 265 lbs. / 120 kg
weight	Digital Analyzer 5s: 150 lbs. / 68 kg
Dimensions (W x D x H)	Prep Station 5s: 35.0 x 26.4 x 24.6 in. / 89 x 67 x 63 cm
	Digital Analyzer 5s: 26 x 26 x 19 in. / 66 x 66 x 48 cm
Power Requirements	100-240 VAC, 610 VAC
Fuse	8A (100-120 VAC) or 4A (200-240 VAC)

# **Environmental Requirements**

Temperature: 18-28°C Humidity: < 80% relative humidity (non-condensing)



# Safety Information (English)

The nCounter Analysis System may only be operated by appropriately trained, professional users. NanoString recommends that all users read and understand this manual prior to attempting to operate the system. Keep this manual in close proximity to the instruments for easy access to instructions and safety information. Failure to comply with the instructions in this manual may pose a dangerous risk to the operator and void the manufacturer's warranty.

- The Digital Analyzer is a Class 1 laser product. The instrument contains an internal Class 2 laser barcode reader. Class 2 laser radiation exposure is possible while the Digital Analyzer cover is opened. Do not stare into the barcode reader laser beam.
- The Prep Station contains high voltage and heater modules indicated by symbols on the deck. Avoid contacting the heater module and electrodes. The instrument is equipped with a safety interlock that will prevent voltage from being applied while the door of the instrument is open.
- Do not use this device in close proximity to sources of strong electromagnetic radiation or vibration, as it may interfere with proper operation.
- Do not attempt to install, move, or disassemble the instrumentation.
- Do not override the door sensors (pinch hazard exists).
- Ensure that all consumables are properly inserted into the system prior to starting.
- Operate the system using only NanoString nCounter reagents in accordance with their indications for use.
- Wear gloves while operating or performing user maintenance on the instrumentation.
- Do not attempt to wash the Prep Station's electrodes or allow water or other solvents to come into contact with the electrodes.
- If it becomes necessary to wash either instrument's touchscreen, use a damp cloth to gently wipe the surface. Do not use excessive water or other solvents, which may damage the screen.

#### **Caution Symbols**

**BIOHAZARD:** Potential hazard from a biological source exists. If you choose to use biohazardous materials on your Prep Station, the instrument may become contaminated with biohazardous materials. Please affix the proper warning labels to your Prep Station if you use biohazardous inputs. Be careful not to touch this area without gloves or other personal protective equipment.

ELECTRICAL HAZARD



CAUTION HOT SURFACE



# Informations de Sécurité (Français)

Le système d'analyse nCounter ne peut être utilisé que par des professionnels dûment formés. NanoString recommande que tous les utilisateurs lisent et comprennent ce manuel avant toute tentative d'utilisation du système. Garder ce manuel à proximité des instruments pour pouvoir accéder facilement aux instructions et aux informations de sécurité. Le non-respect des instructions décrites dans ce manuel peut présenter un risque élevé pour l'opérateur et invalider la garantie du fabricant.

- L'analyseur numérique est un produit laser de classe 1. L'instrument contient un lecteur de codesbarres laser de classe 2 lecteur interne. Une exposition à des rayonnements laser de classe 2 est possible lorsque le capot de l'analyseur numérique est ouvert. Ne pas fixer du regard le faisceau laser d'un lecteur de codes-barres.
- La station de préparation des modules chauffants et haute tension indiquées par des symboles sur le plateau. Éviter tout contact avec le module chauffant et les électrodes. L'instrument est doté d'un mécanisme de verrouillage de sécurité qui empêche l'application de tension pendant que la porte de l'instrument est ouverte.
- Ne pas utiliser ce dispositif à proximité de sources de fortes vibrations ou radiations électromagnétiques, car celles-ci peuvent interférer avec le bon fonctionnement de l'équipement.
- Ne pas essayer d'installer, de déplacer ou de démonter les instruments.
- Ne pas court-circuiter les capteurs de la porte en raison des risques de pincement.
- S'assurer que tous les consommables sont correctement insérés dans le système avant de commencer une procédure.
- N'utiliser le système qu'avec les kits de test nCounter de NanoString et conformément à leur mode d'emploi.
- Porter des gants pendant l'utilisation et la maintenance des instruments.
- Ne pas essayer de laver les électrodes de la station de préparation et éviter que de l'eau ou tout autre solvant entre en contact avec les électrodes.
- Ne pas essayer de laver l'écran tactile des instruments et éviter que de l'eau ou tout autre solvant entre en contact avec les écrans tactiles.

#### **Symboles Attention**

**BIOHAZARD:** Existence d'un danger potentiel provenant d'une source biologique. Si vous choisissez d'utiliser du matériel biologique dangereux sur votre station de préparation, l'instrument peut être contaminé par ce matériel à risque biologique. S' il vous plaît apposer l'étiquetage de mise en garde appropriée à votre station de préparation si vous utilisez des échantillons biologiques dangereux. Faites attention de ne pas toucher cette zone sans gants ou sans autres équipements de protection personnelle.







# **Hardware Overview**

## **General Information**

The instrument serial number and compliance information can be found on the rating plate on the back panel of each instrument (**Figure 1**). Please refer to the rating plate and declaration of conformity (EU only) for compliance information.



Figure 1: Serial number and compliance information for the Prep Station (left) and the Digital Analyzer (right).

Both instruments contain a touchscreen to enable operation of the instrument. The touchscreen enables the user to operate the system by touching a selection on the screen. There are several buttons that appear in the touchscreen user interface. The Main Menu for each instrument is shown in **Figure 2**.



Figure 2: Main Menu on the touchscreens of the Prep Station (left) and the Digital Analyzer (right).

**NOTE**: The Prep Station and the Digital Analyzer are stand-alone units. These instruments do not require connection to a personal computer and are not networked to one another. Connecting the Digital Analyzer to a network is optional and enables data delivery to the user *via* a network connection. Likewise, if the Prep Station is networked, then notifications can be sent to users. The instruments do not communicate with each other.



# **Prep Station**

The Prep Station is a multichannel pipetting robot that processes samples to prepare them for data collection on the Digital Analyzer. The instrument performs liquid transfers, magnetic bead separations, and immobilization of molecular labels on the sample cartridge surface (**Figure 3**).

Prior to placing samples in the Prep Station, samples need to be hybridized according to the protocol outlined in the appropriate user manual (such as the *nCounter XT Assay User* 



Figure 3: The nCounter Prep Station.

Manual (MAN-10023), the *nCounter miRNA Assay Manual* (MAN-C0009), or the *nCounter Elements™ XT* Reagents User Manual (MAN-10086)). On the Prep Station deck, hybridized samples are purified and immobilized in a Sample Cartridge for data collection (**Figure 4**).

All consumable components and reagents required for sample processing on the Prep Station are provided in the nCounter Master Kit and must be loaded onto the Prep Station deck prior to use. No reagent preparation or dilutions are required. The Prep Station can process up to 12 lanes per run in approximately 2.5 to 3 hours depending on which protocol is used.



Figure 4: The Prep Station deck.



# **Digital Analyzer**

The nCounter Digital Analyzer is a multi-channel epifluorescence scanner specifically configured for use with NanoString's nCounter Cartridges. Up to 6 cartridges can be loaded on the Digital Analyzer for sequential processing (**Figure 5**).

It collects data by taking images of the immobilized fluorescent reporters in the sample cartridge with a CCD camera through a microscope objective lens. At the standard data resolution, 280 fields of view (FOV) are collected per flow cell (cartridge lane) yielding data consisting of hundreds of thousands of target molecule counts. The number of images taken corresponds to the number of reporters counted. This determines, in part, the dynamic range and level of sensitivity in the system.

Images are processed internally, and each lane produces one RCC (Reporter Code Count) file containing the counts for that lane. The zipped RCC files are downloaded via USB flash drive and can be imported into the nSolver<sup>™</sup> Analysis Software for QC and analysis. Run data can then be exported as a comma separated values (CSV) format file that can be opened by most commonly used spreadsheet packages, including Microsoft<sup>®</sup> Excel, and can be analyzed using NanoString's nSolver Analysis Software or other data analysis and visualization software packages.



Figure 5: The nCounter Digital Analyzer.



# **Selecting the Instrument Mode**

Users who have the nCounter Dx Analysis System with FLEX configuration are able to run their instruments in either Life Sciences (LS) or Diagnostics (Dx) application modes. The FLEX configuration must be enabled by a NanoString Service Engineer.

**NOTE:** If the FLEX configuration has not been enabled, the instructions provided in this section will not apply; only the Life Sciences application mode will be available. Proceed to *Operating the Prep Station*.

All NanoString Dx assays must be performed using the Diagnostics mode on these instruments. In Diagnostics mode, there are procedures and controls in place that restrict access to data and some features to authorized users. Refer to the *nCounter Dx Analysis System User Manual* (MAN-C0034) for further instruction.



# **Prep Station**

#### Select Instrument Mode

After turning on the Prep Station, the first screen to appear is titled 'Select Instrument Mode' (**Figure 6**). This screen asks the user to select either Diagnostics mode (blue, on the left) or Life Sciences mode (green, on the right).

Press the green icon labeled **NanoString® Life Sciences** to enter Life Sciences mode. The system will load the application and present the Main Menu (**Figure 7**).

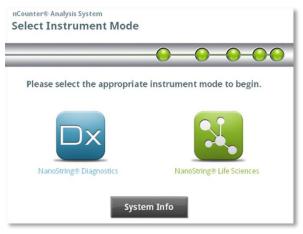


Figure 6: The Prep Station 'Select Instrument Mode' screen.

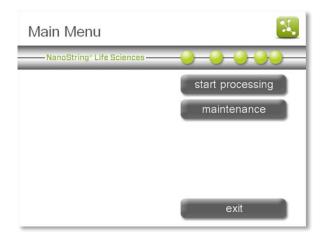


Figure 7: The Prep Station Main Menu in Life Sciences

mode.

#### Switch Instrument Mode

The user may switch between Life Sciences mode and Diagnostics mode from the Main Menu. Press the **exit** button located at the bottom of the Main Menu (**Figure 7**).

The 'Exit Life Sciences Mode' confirmation screen will appear (**Figure 8**).

Press **ok** to exit Life Sciences mode and return to the 'Select Instrument Mode' screen (**Figure 6**). Press **cancel** to return to the Main Menu.

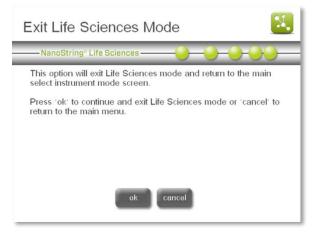


Figure 8: The Digital Analyzer 'Exit Life Sciences Mode' confirmation screen.



# **Digital Analyzer**

#### Select Instrument Mode

After turning on the Digital Analyzer, the first screen to appear is titled 'Select Instrument Mode' (**Figure 9**). This screen asks the user to select either Diagnostics mode (blue, on the left) or Life Sciences mode (green, on the right).



Figure 9:The Digital Analyzer 'Select Instrument Mode' screen.

Press the green icon labeled **NanoString® Life Sciences** to enter Life Sciences mode. If login settings are enabled, a welcome screen will appear, and the user will need to log in by pressing main menu (**Figure 10**; see the section on Enterprise Package settings in the *Operating the Digital Analyzer* section).

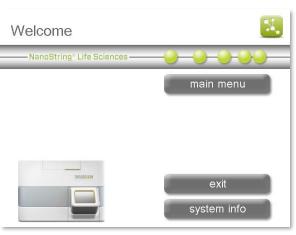


Figure 10: The Digital Analyzer 'Welcome' screen.

After logging in, or if the login settings are not enabled, the system will present the Main Menu (Figure 11). The logout button will only appear if a user is logged in.

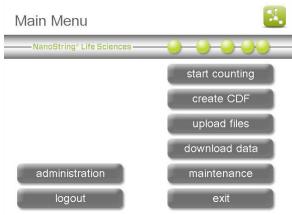


Figure 11: The Digital Analyzer Main Menu in Life Sciences mode.



#### Switch Instrument Mode

The user may switch between Life Sciences mode and Diagnostics mode from the Main Menu. Press the **exit** button located at the bottom of the Main Menu (**Figure 11**).

The 'Exit Life Sciences Mode' confirmation screen will appear (Figure 12).

Press **ok** to exit Life Sciences mode and return to the 'Select Instrument Mode' screen (**Figure 9**). Press **cancel** to return to the Main Menu.

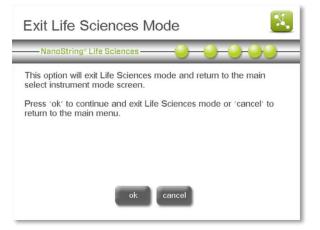


Figure 12: The Digital Analyzer 'Exit Life Sciences Mode' confirmation screen.



# **Operating the Prep Station**

## Prior to Initiating a Run

#### Waste Removal

Prior to starting a new run, ensure that the waste containers have been emptied. Empty waste containers are required for every run.

- 1. Remove the combined waste receptacle by lifting it straight up and out of the Prep Station.
- 2. Remove the liquid waste container from the combined receptacle by using the latch on the front, and dispose of the liquid appropriately.
- 3. Verify that the plastic rack holding the used piercers, tip sheaths, prep plates, and strip tubes from the previous run have all been removed from the deck.

**CAUTION:** If waste containers are not emptied, tips could come into contact with waste liquids and contaminate samples, or excess tips could pile up and cause a system malfunction.

**AVERTISSEMENT:** Si les récipients à déchets ne sont pas vidés, les embouts peuvent entrer en contact avec des déchets liquides et contaminer les échantillons, ou un trop grand nombre d'embouts pourrait s'accumuler et entraîner un dysfonctionnement du système en matière du sécurité.

**CAUTION:** Used plastic ware, such as reagents, cartridges, and pipetting tips, must be collected and disposed of properly in accordance with local safety regulations and laboratory procedures.

**AVERTISSEMENT:** Les récipients en plastique usagés, tels que les plaques de réactifs, les cartouches et les embouts de pipetage, doivent être collectés et mis au rebut correctement selon les procédures du laboratoire et les règlementations locales en matière du sécurité.

**CAUTION:** Do not dispose of biohazardous samples in the sink or other drain. Dispose of all samples in accordance with local safety regulations and laboratory procedures.

**AVERTISSEMENT:** Ne pas verser les échantillons à risque biologique dans l'évier ou autres canalisations sanitaires. Jeter tous les échantillons selon les lois locales de sécurité et les règles de laboratoire.



#### **Consumables Required**

All the consumable items required to run the Prep Station are included in the nCounter Master Kit. This kit contains the reagents and consumables required to process 1, 4 or 16 cartridges depending on the Master Kit size purchased (12-rxn, 48rxn or 192-rxn). No reagent preparation or dilutions are required.

For each cartridge (12 lanes), the components of the nCounter Master Kit include:

- Two nCounter Prep Plates (foil-sealed 96-well plates used by the Prep Station)
- One nCounter Cartridge
- One nCounter Prep Pack
  - Racked Tips and Foil Piercers
  - 12-Tube Strips and Caps
  - Tip Sheaths
  - Cartridge Adhesive Covers
  - Hybridization Buffer



# **Initiating a Run**

The following workflow begins after samples have been hybridized overnight according to the protocol outlined in the appropriate user manual (such as the *nCounter XT Assay User Manual* (MAN-10023), the *nCounter miRNA Assay Manual* (MAN-C0009), or the *nCounter Elements™ XT Reagents User Manual* (MAN-10086)). On the Prep Station deck, hybridized samples are purified and immobilized in a Sample Cartridge for data collection (**Figure 4**).

1. To set up a new run, touch **start processing** from the Main Menu (**Figure 13**).

**NOTE**: The **exit** button will only appear if the user has the nCounter Dx Analysis System with FLEX configuration (see the *Selecting the Instrument Mode* section).

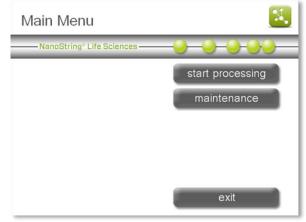


Figure 13: The Main Menu.

- The 'Select Protocol' screen will appear (Figure 14). Select one of three protocols for processing samples:
  - **High Sensitivity** The High Sensitivity protocol increases the binding of all molecules to the slide by about 2-fold vs. prior protocols and adds an extra 30 minutes to the processing time.

**NOTE:** Many Vantage 3D assays specify that the High Sensitivity protocol NOT be used. Please refer to the manual for the assay you are running before selecting this option.

- Standard This is the default run mode.
- Legacy Protocol This mode is consistent with earlier Prep Station software versions and can be used to maintain consistency on long-term projects begun before software updates to Standard (pre-2015).

**NOTE:** All protocols use the same reagents.

Press next.

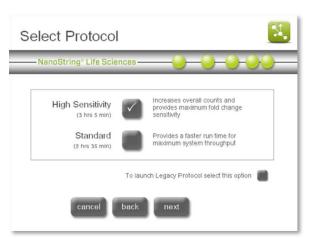


Figure 14: The 'Select Protocol' screen.



 The 'Sample Selection' screen will appear (Figure 15). Select the sample positions that will be processed. Blue tubes will be processed, and grey tubes will not be processed. If processing fewer than 12 tubes, begin with tube 1. Press next.

**NOTE:** Each Prep Plate contains sufficient reagents for 6 lanes. If processing 6 or fewer lanes, use one Prep Plate and ensure the hybridized samples are located in tubes 1-6 and the Prep Plate is placed in the front position. If processing more than 6 lanes, use two Prep Plates. Any number of lanes between 1-12 will still consume an entire Cartridge.

4. The 'Warm Reagents & Cartridge' screen will appear (Figure 16). Cartridges and Prep Plates (Figure 17) must be at room temperature prior to processing. Remove the nCounter Prep Plates from storage at 4°C and the nCounter Cartridges from storage at -20°C. Allow them to equilibrate to room temperature for 10-15 minutes.

**NOTE:** If the cartridges and Prep Plates are not at room temperature prior to use, assay variability may increase. Do not open the cartridge pouch until it has reached room temperature. This will prevent condensation on the cartridge.

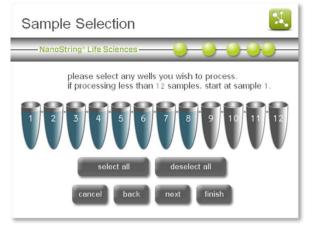


Figure 15: The 'Sample Selection' screen.

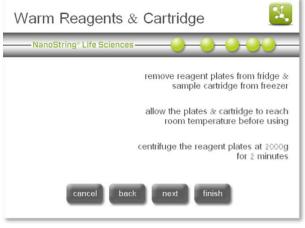


Figure 16: The 'Warm Reagents & Cartridge' screen.





Figure 17: A sealed cartridge (left) and prep plate (right).



Centrifuge the Prep Plates at 2000*g* for 2 minutes to collect all liquids in the bottom of the wells prior to loading the Prep Plates onto the Prep Station deck. After centrifugation, visually inspect plates to ensure that reagents have collected at the bottom of each well. Press **next**.

The 'Waste Receptacles' screen will appear (Figure 18). Remove the liquid waste container from the combined receptacle and dispose of the liquid appropriately. See the previous section on *Waste Removal* for further information.

Tips should be discarded into the appropriate waste stream as outlined in the local organization's laboratory procedures.

If the system is not being used to handle biohazardous samples, liquid waste may be disposed of in the sink or other drain. Press **next**.

6. The 'Reagent Plate' screen will appear (Figure 19). Remove the clear plastic lids and place the Prep Plates on the deck. Make sure plates are oriented with the label facing the operator and that they are flush with the deck surface by aligning the plate on the positioning pin and pressing down firmly near the plate catch (Figure 20). If running 6 or fewer lanes, only the front plate needs to be loaded onto the deck. Press next.

**NOTE:** If the plate is placed incorrectly, the Prep Station will pause the protocol until the user intervenes.

**IMPORTANT:** Do not remove the foil or pierce the wells on the reagent plate. The Prep Station will pierce the wells during processing.

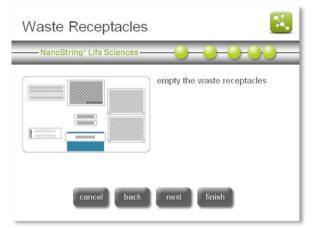


Figure 18: The 'Waste Receptacles' screen.

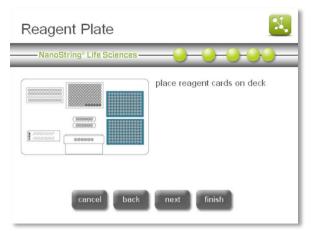


Figure 19: The 'Reagent Plate' screen.

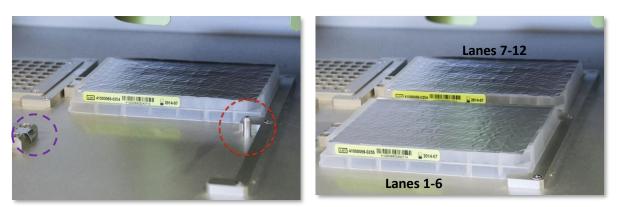


Figure 20: Use the positioning pin (circled in red for the front plate) to ensure correct placement of the Prep Plates. The plate catch (circled in purple) contains a spring clip that ensures the plate is positioned securely. Pressing down on the plate next to this spring clip will ensure the plate is flush with the deck.



7. The 'Tips & Foil Piercers' screen will appear (Figure 21). Remove the metal tip carrier from the Prep Station deck by lifting straight up. Place the tips and the foil piercers into the carrier. It is helpful to place the carrier at eye level to align the plastic tips in the carrier as shown in Figure 22. The shorter, dark grey foil piercers should be in the front as shown.

**IMPORTANT:** There are two sets of tips nested in each box with each side of the box being the opening for one set of tips. Firmly grasp the bottom of the box when opening to avoid accidentally spilling the second set of tips.

Replace the loaded tip carrier back onto the Prep Station deck with the dark grey foil piercers closest to the user.

**NOTE:** Tip sheaths are used to reduce the amount of consumable waste. They allow the system to dedicate tips to a set of 6 samples and store them while processing the other 6 samples.

#### Press **next**.

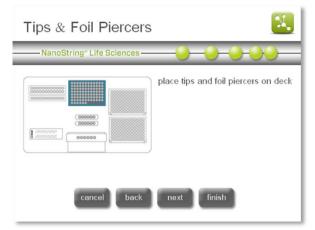


Figure 21: The 'Tips & Foil Piercers' screen.

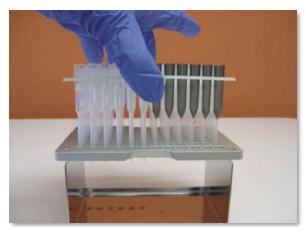


Figure 22: Placing the tips and foil piercers into metal tip carrier.

8. The 'Tip Sheaths' screen will appear (Figure 23).

Place the tip sheaths on the deck and press firmly into place.

Press next.

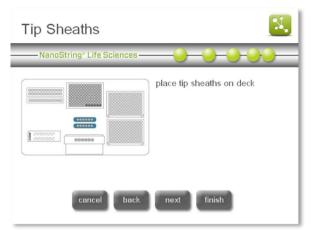


Figure 23: The 'Tip Sheaths' screen.



9. The 'Sample Cartridge' screen will appear (Figure 24). Carefully place a cartridge under the electrode fixture in the orientation shown in Figure 25.

Make sure that it is seated completely in the machined depression. If it is not seated properly, the electrodes may become bent. Press **next**.

Sample Cartridge	<b>N</b> .
NanoString* Life Sciences	-0-0-0-00-
	place sample cartridge on deck
cancel back	next finish



Figure 24: The 'Sample Cartridge' screen.

Figure 25: Insert an unused cartridge into the Prep Station in the orientation shown.

10. The 'Electrode Fixture' screen will appear (**Figure 26**). Carefully lower the electrode fixture in place over the cartridge (**Figure 27**). The 24 electrodes should insert into the 24 wells. Press **next**.

**IMPORTANT:** Do not use the release handle while lowering the fixture. Doing so will prevent the fixture from locking. Press on the body of the fixture away from the release handle (**Figure 27**).

**IMPORTANT:** If any resistance is felt while lowering the fixture, stop and adjust the position of the cartridge slightly. Make sure that the electrodes are correctly aligned. If the electrodes are not aligned, use the 'Align Electrodes' workflow under the Maintenance menu. The Prep Station will not be able to process any of the samples if there are bent electrodes.

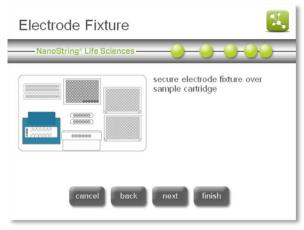


Figure 26: The 'Electrode Fixture' screen.

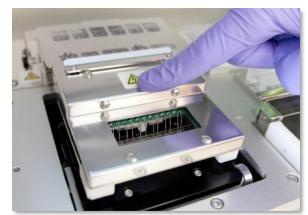


Figure 27: Carefully lower the electrode fixture into place over the cartridge.



 The 'Empty Strip Tubes' screen will appear (Figure 28). Place the empty strip tubes on the deck. Press next.

**IMPORTANT: Only use strip tubes provided by NanoString.** Other tubes have different dimensions and will cause system failure.

Empty Strip Tubes	<b>X</b>
NanoString® Life Sciences	
	place empty strip tubes on deck
cancel back	next finish

Figure 28: The 'Empty Strip Tubes' screen.

12. The 'Hybridized Samples' screen will appear (**Figure 29**). Lift the lid and place hybridized sample strip tube on the deck of the Prep Station, ensuring that tube 1 aligns with position 1 (**Figure 30**). Note that the strip tube is asymmetrically keyed, and if the strip tube is placed incorrectly, the lid won't close properly and the Prep Station will not be able to start processing. Press **next**.

Hybridized sample strip tubes have two notches to ensure proper orientation.

**IMPORTANT:** Ensure that all tube caps are removed from the hybridized samples prior to placing tubes on the deck. Leaving the caps on will result in a pause in the protocol that requires user intervention.

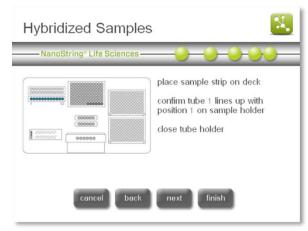


Figure 29: The 'Hybridized Samples' screen



Figure 30: Hybridization sample strip tubes with notched orientation guides.



13. The 'Notification Options' screen will appear (Figure 31).

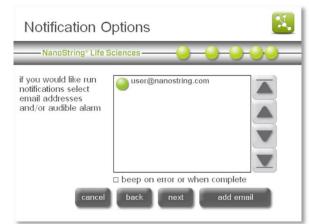


Figure 31: The 'Notification Options' screen.

14. Select the e-mail addresses where error and completion confirmation e-mails should be sent. To add a new address, press add email, type in the address, and press enter (Figure 32). Finally, select whether the Prep Station should make an audible alarm when processing is finished. When all alerts have been set, press next.

Enter email address
NanoString" Life Sciences
$\begin{bmatrix} & & & \\ & & & & \\ & & & \\ & & & & & \\ & & & & \\ & & & & \\ & & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & & \\ & & & & \\ & & & & & \\ & & & & $
Q W E R T Y U I O P
A S D F G H J K L
Trans         Z         X         C         V         B         N         M         enter
× clear alt + del +

Figure 32: The 'Enter email address' screen.



The 'Start Processing' screen will appear (Figure 33). Press start when ready to begin processing.

Start Processing	<b>X</b>
NanoString® Life Sciences	0 0 0 00
	close instrument door
	start
cancel	back

Figure 33: The 'Start Processing' screen.

16. The 'Validating deck layout' screen will appear (Figure 34).

The nCounter Prep Station will first check that all consumables and reagents have been placed properly on the deck. To do this, the Prep Station confirms that the sensors for the sample cartridge, electrode fixture, and heater lid are all in the correct state. The pipette head then checks that tips, tip sheaths, strip tubes, and Prep Plates are all in place by touching them with a set of validation tips. Do not be alarmed that the Prep Station is touching the consumables; this is a part of normal operation. If the Prep Station determines that a

Validating deck layout please wait  😣
NanoString" Life Sciences
current time 8:44 PM run complete 11:07 PM Confirming tips are present
pause

Figure 34: The 'Validating deck layout' screen.

consumable is misplaced, it will instruct the user to adjust the configuration.

The 'Validating deck layout' screen will eventually update to the 'System Processing' screen. Both screens display the current time of day and the estimated time of day that the run will complete. They also provide the option to pause the run.

**IMPORTANT:** It is advised to not abort and re-start the run when there is a deck layout failure. Depending on the progress of the validation process, the Prep Plates may have been pierced and liquid handling may have begun. Reagents may need to be replaced before re-starting the run.

**NOTE:** Please stay with the Prep Station while the deck layout is being validated. Any problems that may be encountered during this step will require user intervention to proceed.



# Pausing or Aborting a Run

The following information is provided should a run need to be paused (optional). If a run does not need to be paused, skip to **Step 18**.

**IMPORTANT:** When a run is paused, resume as quickly as possible. Incubation times are very important, and pausing the instrument can affect data quality and consistency. Runs should be paused only to avoid data loss due to operator error.

17. To pause a run, press **pause**. There may be a delay before pausing while the system waits until it reaches a safe state.

The screen will update to show that the run has been paused (Figure 35).

- a. After pausing, an option to abort the run will appear. Once the hybridized samples have been moved to the Prep Plate for processing they cannot be recovered if the run is aborted.
- **b.** Press **resume** to continue the run, or press abort to end the run.

System Processing	<b>N</b>
NanoString® Life Sciences	
	current time 8:50 PM
. C. Deved for	run complete 11:07 PM
1-6: Paused1sec	
7-12: Paused10sec	
resume	abort

Figure 35: The paused 'System Processing' screen.

**IMPORTANT:** When a run is aborted, the run cannot be restarted. Samples may be lost if any sample processing has already commenced.

 When the run is complete, the blue 'System Processing Complete' screen will appear and the timer will count up (Figure 36). Press next.



Figure 36: The 'System Processing Complete' screen.



- 19. The 'Run Successfully Complete' screen will appear (Figure 37).
- 20. The 'Run Successfully Completed' screen lists the steps to follow once the run is complete, including:
  - a. Remove and discard empty reagent plates.
  - **b.** Remove and discard the empty tip racks and foil piercers.
  - c. Remove and discard the sample strips.
  - d. Remove the sample cartridge and seal the wells.



Figure 37: The 'Run Successfully Completed' screen.

- 21. To release the fixture after the run is complete, press the lever in the center top of the device towards the front with a finger as shown in **Figure 38**.
- 22. After processing is complete, it is important to do the following:
  - a. Seal the wells immediately with the adhesive film provided to prevent evaporation.
  - **b.** Samples should be protected from as much light as possible. Store them in the dark to prevent photobleaching.



Figure 38: Releasing the fixture after a completed run.

- c. Store samples in the refrigerator at 4°C. Once sealed, samples can be stored at 4°C for up to a week with minimal degradation.
- **d.** Empty the waste containers.
- 23. Press finish to return to the Main Menu.



# **Operating the Digital Analyzer**

## **Enterprise Package**

The Digital Analyzer operating system includes an optional Enterprise Package for the creation of user profiles that manage access to instrument functions and data. (The Prep Station does not have this feature.) The Enterprise Package offers the following benefits:

- Option to require that users log in before using the Digital Analyzer, as well as additional configuration options for instrument access.
- Network file transfers between the Digital Analyzer and another computer instead of using a USB flash drive.
- Retrieve audit reports from the Digital Analyzer for tracking and reporting needs.

Once enabled by NanoString, users with the Enterprise Package will see an administration button on the Main Menu (**Figure 39**). Below this there may be a second button to logout depending on the configuration of the instrument.

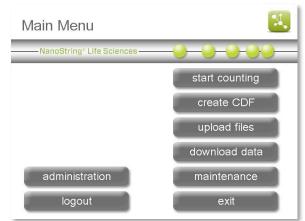


Figure 39: The Main Menu when the Enterprise Package is enabled and an administrator is logged in.

An initial administrator account will be provided when the Enterprise Package is enabled. This administrator will be able to create other user profiles (including additional administrators) and specify access rules for operating the instrument and uploading and downloading data.

**IMPORTANT:** This initial account has the username "admin" and the default password "NanoString!". Set your own password immediately after logging in for the first time.

Press the **administration** button to open the Administration Menu; users will be required to enter a valid administrator username and password.



The Administration Menu will appear (Figure 40).

The Administration Menu provides access to six functions:

- User management Create new user profiles, define user access privileges, and disable existing user accounts.
- Login settings Choose to require that users login before using the instrument.
- USB/FTP settings Establish rules for uploading and downloading data by USB, FTP, and email.
- Archive settings Change preferences for moving or erasing files after download.
- **Cartridge tracking** Set options for tracking nCounter Cartridge barcodes.
- Audit report Generate an audit log on demand.

For more information on the types of files managed by settings in the Administration Menu, see the *Overview of File Structure* section.

# **User Management**

The 'User Management' screen (**Figure 41**) displays a list of all existing user profiles as well as the options to add, update, or delete profiles. User names are displayed in alphabetical order next to a green or red icon. Green icons indicate active user profiles. Red icons indicate user profiles that have been saved but are disabled and do not have access to the Digital Analyzer.

User Management	<u>N.</u>
NanoString® Life Sciences	
jb jdb jane lindy mike add update delete done	

Figure 41: The 'User Management' screen.

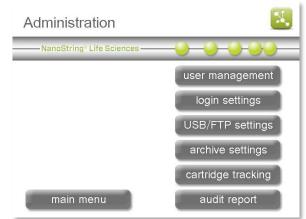


Figure 40: The Administration Menu.

#### Add a User

To create a new user, press the add button.

The 'Create User' screen will appear (Figure 42).

Create User	Create User
NanoString® Life Sciences	NanoString® Life Sciences
user name :   password :   confirm   password :   role :   General     back   save	email address :   display name :   notes :   active :   true (user account active)

Figure 42: The 'Create User' screen. Top half (left) and the bottom half (right)

An asterisk indicates required fields for a new user profile. Scroll between these fields using the arrow buttons on the right.

- User Name Enter a unique user name, which will be used during login and to identify users on the User Management screen.
- Password/Confirm Password Enter a password for the user.
- Role Users can be identified as "General" or "Administrator." Administrators have access to the Administration Menu (Figure 40) and can create other user profiles.
- Email address Enter the e-mail address that will be associated with the User.
- Display Name The display name will appear when selecting email recipients for instrument alerts and sending data. Unlike the user name, it does not need to be unique.
- Notes Optional notes may be added to the user profile and will only be visible to administrators.
- Active Toggle this field between "true (user account active)" and "false (user account disabled)." Disabled profiles will not have access to the Digital Analyzer, but the profile information will remain saved.

When finished, press **save** to create the new user profile, or press **back** to return to the User Management screen without creating a profile.



#### Update or Disable User

To update an existing user profile, select that profile and press the **update** button (**Figure 41**). The user information will appear, very similar to the process of creating a new user. Select and edit fields as necessary, then press the **save** button. Press **back** to return to the 'User Management' screen without making any changes.

To disable an existing user profile, select that profile and press the **delete** button (**Figure 41**). The green circle next to the user profile will change from green to red, and the user profile can be reactivated later by using the update function. See the user "jdb" in **Figure 41** as an example. Be aware that user profiles can never be truly deleted, as this would invalidate the audit trail.

#### **Login Settings**

The 'Login Settings' screen (**Figure 43**) enables the administrator to require that users log in before using the Digital Analyzer. Press the login button to toggle this field between "enabled (password protected)" and "disabled."

These login settings can be adjusted. Press the timeout button to change the time required before the system locks the screen due to inactivity. For example, a two-minute timeout will allow the instrument to wait two minutes without receiving new commands. Set the timeout to **zero** to disable the timeout. Be aware that the screen will lock automatically when a new run begins if the login setting is enabled. Users can also lock the screen by logging out.

Login Settir	igs 🔣
NanoString® Lif	e Sciences OOOO
login :	enabled (password protected)
timeout :	2 minutes
	set to 0 to disable timeout
	back save

Figure 43: The Login Settings screen.



#### **USB/FTP Settings**

The 'USB/FTP Settings' screen (**Figure 44**) enables the administrator to choose which data transfer options are available to upload RLFs and CDFs and to download RCC files.

For more information on these files and their definitions, see the *Overview of File Structure* section. For more information on how to network the instrument, see the section on System Setup in *Technical Support and Maintenance*. File transfer options include USB flash drive, FTP, and email. However, email is only used to download RCC files and cannot be used to upload files to the instrument.

Select each field to change that setting, choosing one or more file transfer options for each file type. Selecting the same field repeatedly will toggle between options. If desired, the **RCC checksum** option can create a separate checksum file stored in the same directory as the RCC file. This checksum can be used to check that the RCC file was not altered during file transfer.

USB/FTP Settings		X.
NanoString® Lif	e Sciences	)—
RLF upload :	Both USB and FTP	
CDF upload :	Both USB and FTP	
RCC download :	USB, FTP and Email	
RCC checksum :	none	
	back save	



#### **Archive Settings**

The 'Archive Settings' screen (**Figure 45**) controls two functions that enable users to move or delete files on the Digital Analyzer.

The Digital Analyzer is normally configured to move RCC files to an 'Old Data' folder when they are downloaded after a run. Some users may prefer to keep all RCC files in a single location, e.g., when downloading files by FTP. Press the **move older data** button to enable or disable this feature.

The instrument may also accumulate a variety of other files after prolonged use. The Disk Cleanup feature is normally used to check the available disk space and also to delete RCC files, CDFs, and RLFs (see the section on Disk Cleanup in *Technical Support and Maintenance*). Press the **disk cleanup** button to enable or disable this feature.

Archive Settings
NanoString® Life Sciences
move older data : do not move after download disk cleanup : disabled
back save

Figure 45: The **Archive Settings** screen.



#### **Cartridge Tracking Settings**

The 'Cartridge Tracking Settings' screen (Figure 46) allows the administrator to enable two features that track which nCounter Cartridge barcodes have been run on the Digital Analyzer. The first, audit log, instructs the instrument to save the cartridge barcode for inclusion in future audit reports (see the next section). The second, RCC file, will store the cartridge barcode in the RCC file generated during data collection. Both options can be toggled between 'True' and 'False'.

Cartridge Tracking Settings	X.
NanoString® Life Sciences	
audit log : True RCC file : True back save	
back save	

Figure 46: The Cartridge Tracking Settings screen.

NOTE: The cartridge barcode scanner will be disabled when both audit log and RCC file are set to "False."

#### **Audit Report Generation**

The 'Audit Report Generation' screen (**Figure 47**) enables the administrator to create an audit report with information on files created, deleted, and transferred, runs performed, configuration changes, and which users accessed the instrument to perform these actions.

Use the number pad to enter the start and end dates for the audit period. Then press the destination button to choose where the audit report will be saved. It can be stored on the FTP server (within the RCCData folder) if the instrument is networked, or it can be saved to a USB flash drive. Finally, press **generate** to create and save the audit report.

Audit Report Generation	on			<b>X</b>
NanoString® Life Sciences				<del>.</del>
Start Date         (mm/dd/yyyy)           05         /         17         /         2015	7	8	9	
End Date (mm/dd/yyyy)	4	5	6	
06 / 17 / 2015	1	2	3	
destination : FTP	0	del	ete	
back			gene	rate

Figure 47: The Audit Report Generation screen.



# **Overview of File Structure**

There are three types of files used by the nCounter Analysis System: the Cartridge Definition File (CDF), the Reporter Library File (RLF), and the Reporter Code Count (RCC) file.

- The Cartridge Definition File is created by the user. It defines assay-specific data to associate with the data output and the parameters used by the Digital Analyzer during image collection and processing.
- The Reporter Library File is generated by NanoString and is unique to each custom CodeSet. It contains the information used during image processing to assign target identities to the barcodes.
- The Reporter Code Count file is generated by the Digital Analyzer. Each one contains the data for one of the twelve lanes (assays) in a cartridge, detailing the number of counts for each target in an assay.

Each CodeSet ships with a USB flash drive containing the following files for use on the Digital Analyzer:

- Three read-only folders for Cartridge Definition Files (CDFData), Reporter Library Files (RLFData), and Reporter Code Count files (RCCData) (Figure 48).
- A CDF template.
- A compressed file containing the nSolver Analysis Software installer for Microsoft<sup>®</sup> Windows<sup>®</sup>, a PDF copy of the nSolver Analysis Software Manual, and a folder containing sample RCC files.

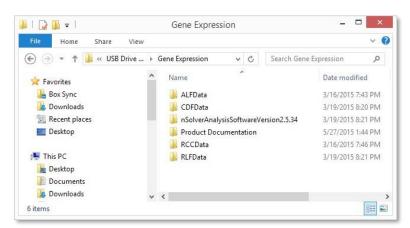


Figure 48: Example file folders stored on a USB flash drive.

- A compressed file containing the nSolver Analysis Software installer for Macintosh<sup>®</sup>, a PDF copy of the nSolver Analysis Software Manual, and a folder containing sample RCC files.
- Product-specific assay manuals, product inserts, and data analysis guides in PDF format.
- The nCounter Analysis System User Manual.
- Material Safety Data Sheet(s).

Users may provide their own USB flash drive to transfer files but must create the same three folders in order to interface with the nCounter system.

**NOTE:** The USB flash drive and the Digital Analyzer have been designed specifically to enable quick and accurate file transfers. Users may create sub-folders for CDFs to facilitate file organization. However, do not create sub-folders for RLFs or RCC files. If a user creates sub-folders within the RLF folder on a USB flash drive, the system will not be able to locate the RLFs.

**NOTE:** nCounter TagSets do not ship with a USB flash drive; the RLF is provided via email.



#### **Navigating File/Folder Screens**

The file/folder structure is set up similar to a personal computer with nested folder structures. Navigate down the folder hierarchy by "double- touching" the folder to open it, similar to double-clicking when using a mouse on a computer (**Figure 49**). To navigate back to a higher-level folder, double-touch the root directory to return to the top level.

Use the up and down single arrow keys to scroll up and down within a window one file at a time. Use the up and down double arrow key (those that have lines at the top or bottom) to go to the very top or the very bottom of the file list.

Select CDF Destination
NanoString* Life Sciences
LI Folder
cancel back next new folder

Figure 49: Navigating files and folders from the Digital Analyzer display.

### **Reporter Library Files**

Users need only upload Reporter Library Files (RLFs) when they first receive a new CodeSet. If the Enterprise Package is configured, RLFs may also be transferred automatically by FTP (certain configurations may disable transfer by USB flash drive). Once an RLF has been uploaded, it will always be available until it is manually deleted from the Digital Analyzer. The RLF corresponding to a specific CodeSet must be uploaded before setting up a run using that CodeSet. Instructions for deleting RLFs are in *Technical Support and Maintenance*.

Upload RLFs via USB Flash Drive

- 1. Insert the USB flash drive into the USB port on the right side of the display.
- From the Main Menu, press upload files (Figure 50).

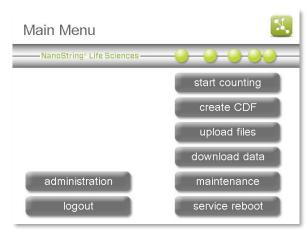


Figure 50: The Main Menu.

**NOTE:** The **exit** button will only appear if the user has the nCounter Dx Analysis System with Flex configuration (see *Selecting the Instrument Mode*).



The 'Upload Files' screen will appear (Figure 51). Select upload RLF.

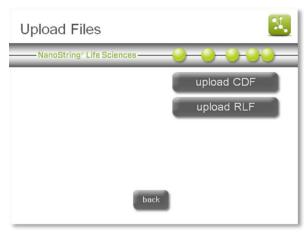


Figure 51: The 'Upload Files' screen

The 'Select RLF Source' screen will appear (Figure 52).

Those folders and RLFs found on the USB flash drive that have not already been uploaded will be displayed. Select one or more files and press **next** to upload the file(s).

The RLF will be saved on the Digital Analyzer.

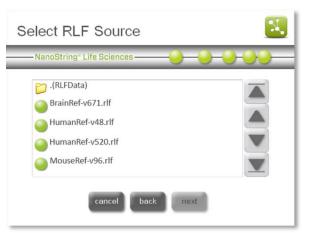


Figure 52: The 'Select RLF Source' screen



# **Upload RLFs via FTP**

Acquire the IP address and FTP password prior to completing the following instructions to transfer data from a personal computer to the Digital Analyzer. See *Digital Analyzer Maintenance* for more information.

If the instrument FTP settings have been configured, RLFs may be transferred directly to the Digital Analyzer from a personal computer. There are many FTP applications available, any of which should work for transferring data between the Digital Analyzer and a personal computer.

1. On a Windows computer, open Windows Explorer or File Explorer. On a Macintosh computer, open Finder and select **Connect to Server...** from the Go drop-down menu. Once the file manager is open, type **FTP://(IP\_address\_here**) as shown in **Figure 53**.

🖳   🔂 📗 = I	This PC	- • ×		
File Computer View		~ 🕜		
	✓ C Search This PC	Q	● ● ○ Con	nect to Server
🔆 Favorites	<ul> <li>Folders (6)</li> </ul>	^	Server Address:	
🕌 Box Sync 🐞 Downloads	Desktop		ftp://10.0.0.117	+ @~
📃 Recent places			Favorite Servers:	
Desktop	Documents	- 11		
📲 This PC				
📜 Desktop 朜 Documents	Downloads			
Downloads Marcom Music	Music	- 1	? Remove	Browse Connect
Pictures Videos	Pictures			
Local Disk (C:)	v	8== ==1		

Figure 53: Enter the Digital Analyzer's IP address in File Explorer (left) or Finder (right).

2. Press Enter. The FTP log on window will appear (Figure 54).

		Log On As	
۲	accepted. FTP server: User name: Password: After you log o	er does not allow anonymous logins or the e-mail address was not 10.0.0.117 technician on, you can add this server to your Favorites and return to it easily. encrypt or encode passwords or data before sending them to the tect the security of your passwords and data, use WebDAV instead.	Enter your name and password for the server "10.0.0.117". Connect as: Guest Registered User Name: technician Password: ••••••• Remember this password in my keychain
	Log on <u>a</u> no	nymously Save password	Cancel Connect

Figure 54: The FTP log on window using File Explorer (left) or Finder (right).



3. Enter 'technician' as the FTP user name, enter the password as defined in the maintenance menu, and then click **Log On** or **Connect**.

The contents of the instrument server will appear (Figure 55).

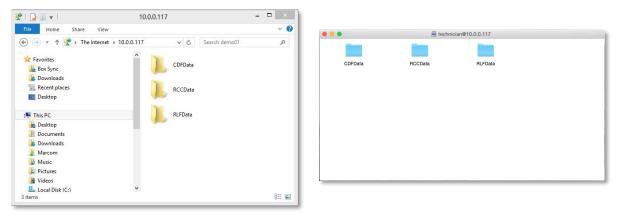


Figure 55: The contents of the instrument server when using File Explorer (left) or Finder (right).

4. Open the RLFData folder.

The RLFData folder will open and display the available files (Figure 56).

🍑   🎧 📑 🗢   RLFDat	ta –	×				
File Home Share View		v 🔞				
	✓ 🖒 Search RLFData	Q		RLFData	_	
<ul> <li>★ Favorites</li> <li>▲ Box Sync</li> <li>֎ Downloads</li> <li>20 Recent places</li> <li>■ Desktop</li> </ul>	n6_vnTrain48.tff n6_vRCC16.tff	^		exec		
👰 This PC	TestRLF1.rlf			n6_vnTrain48.rlf		
Desktop			Name	Date Modified	Size	Kind
Documents			n6_vnTrain48.rlf	Oct 24, 2014, 12:00 AM	12 KB	Unix Ele File
Downloads			n6_vRCC16.rlf	Oct 24, 2014, 12:00 AM	2 KB	Document
Marcom			TestRLF1.rlf	Jun 8, 2015, 12:05 PM	2 KB	Document
Marcon Marcon						
j Pictures						
🗎 Videos						
Local Disk (C:)		~				
3 items		833 🛋				

Figure 56: The contents of the 'RLFData' folder when using File Explorer (left) or Finder (right).

5. Copy and paste RLFs from the personal computer into the RLFData folder.



# **Cartridge Definition Files**

Cartridge Definition File (CDF) defines sample-specific data to associate with the data output and defines the parameters the Digital Analyzer will use during image collection and processing. Data contained in the CDF include:

Data Field	Description
Sample Identifier (Sample ID)	<b>Required.</b> Sample identifiers can be specific names or assigned default names 01-12 corresponding to one or more of the 12 flow cells on the sample cartridge. The sample ID is displayed as part of the Reporter Code Count (RCC) file name and as a column heading in the data output file.
Owner Identifier (Owner ID)	<b>Optional.</b> Example uses include the name of individual running the experiment or the study's Principle Investigator. Details are output within the RCC file as general information.
Comments	<b>Optional.</b> Example uses include details regarding the project or a specific sample. Details are output within the RCC file as general information.
Reporter Library File (RLF)	<b>Required.</b> The RLF contains the code key information used during image processing to assign target identities to the barcodes. If this file is not designated, the system will not allow data collection. Each RLF is unique to a custom CodeSet, so the correct RLF is critical.

**IMPORTANT:** The version of the RLF must match the version of the CodeSet used in the hybridization.

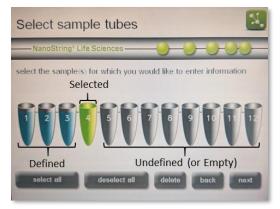
Users can create a new CDF on a personal computer or directly on the Digital Analyzer using the touchscreen. NanoString recommends users create CDFs on their computer if detailed information for each sample is required. In this case, a USB flash drive can be used to transfer the CDF to the imaging instrument. However, if the Enterprise Package is enabled, some configurations may require users to transfer the CDF via FTP (see the *Enterprise Package* section).

When on the 'Select Sample Tubes' screen (**Figure 57**), tubes will be shown in three different colors.

Blue – Defined. Blue tubes have the required information associated with them.

Green – Selected. Green tubes are currently selected.

**IMPORTANT:** When multiple tubes are selected, *all of the info* showing on the screen will <u>overwrite</u> the existing info for **all** *tubes selected*. If you are not using a CDF, it is recommended that you begin with all tubes selected to input the common info (such as FOV), then select each tube separately to apply sample-specific information.





Grey – Undefined (empty). Grey tubes have no information associated with them, either because they haven't been selected yet, or they are empty.



Use cases vary, so flexibility was built into the CDF creation process to accommodate many different situations. Some example scenarios are described below.

- Scenario 1: Create a CDF on a personal computer and transfer the file to the Digital Analyzer. This is the most practical scenario if detailed and unique information is required for each sample within a cartridge. See *Creating a CDF on a Personal Computer*.
- Scenario 2: Create a CDF on the Digital Analyzer and enter data separately for each tube. This may be required if each sample uses a different CodeSet and required a different RLF, or if the user requires unique information for each sample. This is the most time intensive scenario. See *Creating a CDF on the Digital Analyzer*.
- Scenario 3: Create a CDF on the Digital Analyzer and enter data for multiple tubes simultaneously. See *Creating a CDF on the Digital Analyzer*.
- Scenario 4: Create a CDF on the Digital Analyzer, enter data for all tubes, and modify information for one tube. This scenario would be used if most, but not all, data were the same for samples in a single cartridge. See *Creating a CDF on the Digital Analyzer*.

# **Creating a CDF on a Personal Computer**

Each USB flash drive that comes with a new CodeSet has a CDF template loaded onto it in the folder titled "CDFData". If you got your RLF in an e-mail from Bioinformatics, that e-mail will also contain the CDF template that comes on the USB drive for CodeSets.

Users can modify this CDF template to contain personalized sample information; it can be imported and modified in Microsoft Excel or Notepad for Windows (Textedit for Macintosh). Depending on a user's system setup, it may also be necessary to enable the display of file type extensions. Follow the instructions provided below.

#### Display File Type Extensions

- 1. Open File Explorer and select the Options from the View menu (Figure 58).
- 2. Under the **Options** menu, select **Change folder** and search options.

= 1 🗋 🗍 =	1	This PO	C			- 🗆 🛛
File Cor	mputer View					^ <b>(</b>
Navigation pane +	Contraction of the second s	E Details	Current view •	Show/	Options	
( → → → → Favorites	↑ 🖳 ד This Po	A Folders (6)	Y (	0 0	earch This PC	م.
Herent Box Sy Box Sy Box Sy Box Sy	oads	Desktop				
🚺 Downl	oads t places	Desktop	nts			
Downl 强 Recent	oads t places pp pp nents					

Figure 58: Location of the Options button.

- The 'Folder Options' window will appear (Figure 59).
   Select the View tab and uncheck the option Hide extensions for known file types.
- 4. Click OK.

Folder Options ×	
General View Search	
Folder views You can apply this view (such as Details or Icons) to all folders of this type. Apply to Folders Reset Folders	
Advanced settings:	
<ul> <li>Always show icons, never thumbnails</li> <li>Always show menus</li> <li>Display file icon on thumbnails</li> <li>✓ Display file size information in folder tips</li> <li>Display the full path in the title bar</li> <li>Hidden files and folders</li> <li>Don't show hidden files, folders, or drives</li> <li>Show hidden files, folders, and drives</li> </ul>	
<ul> <li>✓ Hide empty drives</li> <li>→ Hide extensions for known file types</li> <li>✓ Hide folder merge conflicts</li> </ul>	
Restore Defaults	
OK Cancel Apply	

Figure 59: The 'Folder Options' window.



#### Modify an Existing CDF in Excel

1. Available characters for renaming a file include: @ ^ + - # % ( ~ & )

**NOTE:** Filenames of CDFs cannot contain any characters in the filename not displayed on the touchscreen keypad. Characters such as underscore cannot be used.

- 2. Append the extension ".csv" to the end of the file name.
- 3. Double-click the file to open it in Excel.
- 4. Fill in the CDF as shown in Figure 60.

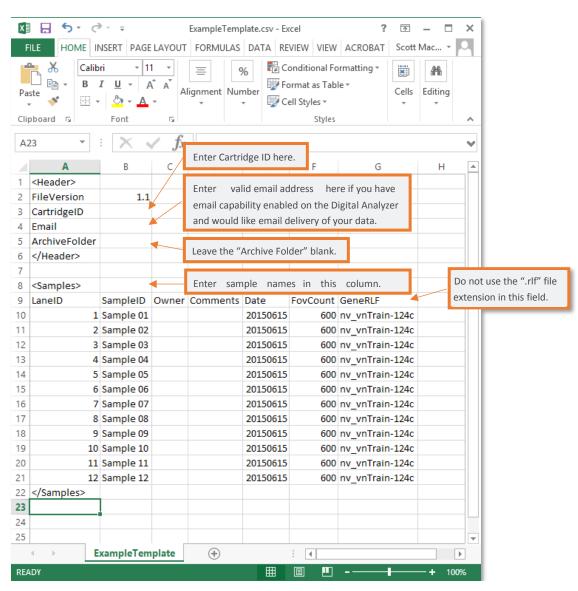


Figure 60: A sample CDF viewed in Excel. Enter information for one assay in each row.



Column definitions:

- LaneID The LaneID column defines which the flow cells in the cartridge will be scanned. If all twelve lanes will be scanned, this should not be changed. If only a subset of lanes will be scanned, the information for the empty lanes can be deleted.
- Sample ID This column is where the user may name individual samples; technical replicates may be assigned the same name.
- **Owner** This is an optional field that can be named; information is output with the data.
- **Comments** Enter additional sample or experimental details in the Comments field; information is output with the data.
- **Date** The Date information is optional and can contain any date in the format **YYYYMMDD**. The date of a scan is automatically added to the beginning of the RCC file name, so it is not required to be included here.
- **FOVCount** This field specifies the number of images (fields of view) to analyze per assay, which corresponds to the amount of data to collect. Options are 555, 280, 100, or 25.
- **GeneRLF** This field defines the reporter library file to associate with the data. It is extremely important that this filename be correct or data will be misinterpreted. The ".rlf" file type extension should NOT be used here.
- When finished entering the information, save the file as a ".csv" file type; the file name will include BOTH ".cdf" and ".csv" extensions as shown in Figure 61 (top).
- 6. Close the file.
- Remove the extra ".csv" file extension, leaving the file extension ".cdf" (Figure 61, bottom). If a warning appears, click Yes to continue.

**NOTE:** The CDF must be placed in the folder on the USB flash drive entitled CDFData in order for the Digital Analyzer to find it.

riie na	me: ExampleT	emplate.cdf.csv			
Save as t	ype: CSV (Com	nma delimited) (*.csv)			
🗋 🗐 =		Docu	uments		×
File Home	Share	View			<u>^</u>
avigation pane •	Extra large Medium ice List	icons 🔊 Large icons ons 🔅 Small icons	Current view •	Show/ Options	
Panes		Layout			
€	順 🕨 This P	C ▶ Documents	Y C	Search Docum	ents 🔎
☆ Favorites			Ren	ame	
Box Sync Downloa	12		a file name exter you want to chan	nsion, the file might b nge it?	
Desktop				Yes	No
		T Desktop	17.	11	/4/2014 4:35 PM
📔 Desktop					

Figure 61: Removing the ".csv" file extension.



# Modify an Existing CDF in Notepad

- 1. Open the CDF in Notepad (or Textedit). Note that all the information present in the Excel spreadsheet shown in **Figure 59** is also visible in Notepad (**Figure 62**).
- 2. Enter or change the information appropriate for the experiment:
  - Under <Header>

Do not alter any of the existing text. Enter the cartridge ID and user e-mail address after the comma. Do not use spaces.

• Under <Samples>

Do not alter the definitions in the first line (LaneID, SampleID, Owner, Comments, Date, FOVCount, GeneRLF). Rather, enter information on a new line. The values for these definitions must be separated by commas. Do not use spaces.

	_	×
ExampleTemplate.cdf - Notepad		~
File Edit Format View Help		
<pre><header> Fileversion,1.1 CartrdigeID, Email, ArchiveFOlder, </header> LaneID,SampleID,Owner,Comments,Date,FovCount 1,Sample 01,,20150615,600,nv_vnTrain-124c 2,Sample 02,,20150615,600,nv_vnTrain-124c 3,Sample 03,,20150615,600,nv_vnTrain-124c 4,Sample 04,,20150615,600,nv_vnTrain-124c 5,Sample 05,,20150615,600,nv_vnTrain-124c 6,Sample 06,,20150615,600,nv_vnTrain-124c 7,Sample 07,,20150615,600,nv_vnTrain-124c 8,Sample 08,,20150615,600,nv_vnTrain-124c 10,Sample 10,,20150615,600,nv_vnTrain-124c 11,Sample 11,,20150615,600,nv_vnTrain-124c 12,Sample 12,20150615,600,nv_vnTrain-124c 13,Sample 11,,20150615,600,nv_vnTrain-124c 14,Sample 11,20150615,600,nv_vnTrain-124c 14,Sample 11</pre>		^
12,Sample 12,,,20150615,600,nv_vnTrain-124c <samples></samples>		
(Sumpress		$\sim$
<		>

Figure 62: A sample CDF viewed in Notepad.

**IMPORTANT:** All of the commas in this file are necessary to separate data fields and should not be removed. If deleted, the file will not be read properly.

3. Save the file with a ".cdf" extension.



# **Upload CDFs via USB Flashdrive**

Users can upload a CDF created on a personal computer to the Digital Analyzer from a USB flash drive. If the Enterprise Package is configured (see the *Enterprise Package* section), CDFs may also be transferred by FTP, or the USB transfer option may be disabled.

- 1. Insert the USB flash drive containing the CDF saved in the **CDFData** folder into the USB port on the side of the touchscreen.
- 2. From the Main Menu, press **Upload Files**. The 'Upload Files' screen will appear (**Figure 63**).

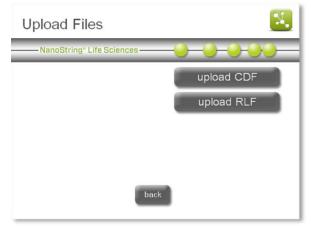


Figure 63: The 'Upload Files' screen.

- 3. Select **upload CDF**. The 'Select CDF Source' screen will appear (**Figure 64**).
- 4. Browse to the location on the USB flash drive where the CDF is stored and select the CDF. Press **next**.

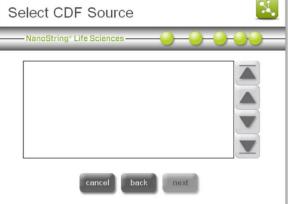


Figure 64: The 'Select CDF Source' screen.

 The 'Select CDF Destination' screen will appear (Figure 65). Browse to the folder location on the analyzer where the CDF should be saved. Press save.

Select CDF Destination	N,
NanoString® Life Sciences	)
Destination: CDFData cancel back next new folder	

Figure 65: The 'Select CDF Destination' screen.



#### **Upload CDFs via FTP**

Acquire the IP address and FTP password prior to completing the following instructions to transfer data from a personal computer to the Digital Analyzer. See *Digital Analyzer Maintenance* for more information.

If the instrument FTP settings have been configured, RLFs may be transferred directly to the Digital Analyzer from a personal computer. There are many FTP applications available, any of which should work for transferring data between the Digital Analyzer and a personal computer.

1. On a Windows computer, open Windows Explorer or File Explorer. On a Macintosh computer, open Finder and select **Connect to Server...** from the Go drop-down menu. Once the file manager is open, type **FTP://(IP\_address\_here**) as shown in **Figure 66**.

🖷   🔂 🖟 🕈 I	This PC –	
File Computer View		~ <b>0</b>
🔄 🄄 – 🕆 🕵 ftp://10.0.0.117/	V 🖒 Search This PC	P Connect to Server
😭 Favorites	Folders (6)	A Server Address:
🕌 Box Sync		
🐌 Downloads	Desktop	ftp://10.0.0.117 + @~
📃 Recent places		Favorite Servers:
E Desktop	Documents	
🜉 This PC		
📔 Desktop	Downloads	
Documents		
〕 Downloads		
Marcom	Music	? Remove Browse Connect
🚺 Music	Area .	Remove     Browse     Connect
崖 Pictures		
📕 Videos	Pictures	
Local Disk (C:)	~	v
17 items		

Figure 66: Enter the Digital Analyzer's IP address in File Explorer (left) or Finder (right).

2. Press Enter. The FTP log on window will appear (Figure 67).

				Log On As	×
10.0.0.117	ame and password for the server ".	accet	er the server does no pted. server: 10.0.0.1	t allow anonymous logins or the e-mail address was no	t
Connect as:	Guest Registered User	User	mame: technic	ian V	
	technician			add this server to your Favorites and return to it eas encode passwords or data before sending them to the ecurity of your passwords and data, use WebDAV inste	· ·
	ber this password in my keychain Cancel Connect		.og on <u>a</u> nonymously	Save password	

Figure 67: The FTP log on window using File Explorer (left) or Finder (right).



3. Enter 'technician' as the FTP user name, enter the password as defined in the maintenance menu, and then click **Log On** or **Connect**.

The contents of the instrument server will appear (Figure 68).

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		earch demo01	Q			
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Recent places	RCCData					
🌉 This PC	RLFData					
Documents						
Marcom						
<ul> <li>Pictures</li> <li>Videos</li> </ul>						
Local Disk (C:) 3 items	~	83	= =			

Figure 68: The FTP log on window using File Explorer (left) or Finder (right).

4. Open the **CDFData** folder.

The CDFData folder will open and display the available files (Figure 69).

4444	CDFData	•••	h CDFData	✓ 🖒 Search CDFDat	🔄 🄄 🔻 🏌 퉬 « 10.0.0.117 🕨 CDFData
			~		
		ex		longID.cdf	Favorites  Four Favorites  Box Sync  Downloads
				longID112.cdf	Recent places
				longio rizicui	E Desktop
	longID.cdf			longID128.cdf	1 This PC
Size Kind	Date Modified	Name			📔 Desktop
	Jun 23, 2015, 12:13 PM	longID.cdf			Documents
	Jun 23, 2015, 12:24 PM	longID64.cdf		longlD64.cdf	
281 bytes Document	Jun 23, 2015, 12:27 PM	longID65.cdf			Ju Downloads
	Jun 23, 2015, 12:26 PM	longID96.cdf			Marcom
					Music
328 bytes Document	Jun 23, 2015, 12:23 PM	longID112.cdf		long/D65.cdf	
328 bytes Document 344 bytes Document	Jun 23, 2015, 12:23 PM Jun 23, 2015, 12:22 PM Jun 23, 2015, 12:19 PM	longID112.cdf longID128.cdf longIDminus.cdf		longID65.cdf	Pictures
	OngID.cdf     Date Modified	Name	- 1	longID128.cdf	🜉 This PC

Figure 69: The contents of the 'CDFData' folder when using File Explorer (left) or Finder (right).

5. Copy and paste CDFs from the personal computer into the CDFData folder.



#### Creating a CDF on the Digital Analyzer

The most rapid method to speed data entry on the Digital Analyzer is to create a template CDF for each CodeSet. To accomplish this, the user would select all tubes, use default sample IDs of 01-12, select an RLF, and press done on the confirmation screen. Save the new CDF with the CodeSet name as the file name.

This template CDF can be used for any sample cartridge that is used in conjunction with the CodeSet defined in selected RLF. However, the user will need to track samples, as no unique identifying information besides the sample cartridge ID will be contained in the data output.

The instructions below will discuss the entire process of created a CDF on the Digital Analyzer, including how to input unique identifying information for each sample.

- From the Main Menu (Figure 50), press create CDF. The 'Cartridge information' screen will appear (Figure 70).
- 2. Press cartridge ID to enter a cartridge ID.

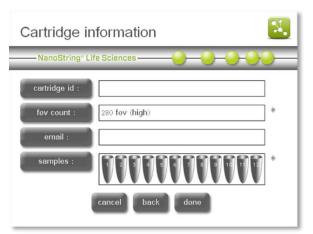


Figure 70: The 'Cartridge information' screen.

- The 'Enter cartridge ID' screen will appear (Figure 71).
- 4. Enter the cartridge ID using the touch screen keyboard and press enter when done. The cartridge id field is optional in the 'Create CDF' workflow. If left empty, the user will be prompted to enter the cartridge ID after selecting the CDF in the 'Start Counting' workflow.

Enter cartridge id	cancel enter
——NanoString*Life Sciences———————————————————————————————————	-0-0-
MyCartridge	
	8 9 0
	IOP
A S D F G H J	KL
	M <u>enter</u>
clear alt	← + del -

Figure 71: The 'Enter cartridge ID' screen.

- The Digital Analyzer will return to the 'Cartridge information' screen (Figure 72).Press fov count to change the data resolution.
- 6. The 'Select Data Resolution' screen will appear (Figure 73).

Select the desired data resolution. Press enter.

The number of images (fields of view, or FOVs) collected corresponds to the sensitivity and dynamic range that can be achieved. If a higher throughput is preferred on the analyzer, the user may opt to collect fewer images if sensitivity is less important. For example, rescanning a cartridge to check for data quality at low resolution takes approximately 2.5 minutes per flow cell *vs.* 20 minutes per flow cell at maximum resolution.

- **low** resolution takes 25 images per sample
- medium resolution takes 100 images per sample
- high resolution takes 280 images per sample
- very high resolution takes 490 images per sample
- max resolution takes 555 images per sample

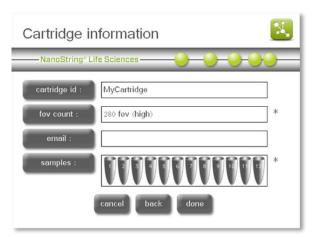


Figure 72. The Cartriage information screen							
Select Data Resolution							
NanoStr	ing® Life Science	es					
dat	a resolution: low med	280 FOVs Please select the data resolution for this cartridge. Higher resolutions have longer scan times.					
	high very high max						
L	b	ack enter					

Figure 73: The 'Select Data Resolution' screen

**NOTE:** Specifications for this system are based on the "high" data resolution setting. Therefore, the "very high" and "max" resolution settings do not guarantee that the throughput specification will be met.

7. The system will return to the 'Cartridge Information' screen (Figure 72).



8. Press **email** to select an email address; if the Enterprise Package is enabled, some configurations may disable email notifications.

The 'Select email address' screen will appear (Figure 74).

Select an email address where the data from a cartridge will be sent. If the Enterprise Package is enabled, then the display name will appear next to each email address and users will have the option to select more than one email address.

Data will be stored in the 'older data' section of the Digital Analyzer after sent by email and can be retrieved using a USB flash drive or FTP if the instrument is correctly configured.

**NOTE:** Email must be configured first in order for email notifications to function. Selecting an email address from this screen may be possible, but no results will be sent if the configuration is incorrect. To adjust email settings, see the section on *System Setup*.

- 9. Press **enter**. The system will return to the 'Cartridge information' screen (**Figure 75**).
- 10. Select the **samples** field. The 'Select sample tubes' screen will appear (**Figure 76**).



Figure 74: The 'Select email address' screen.

Cartridge in	formation	<b>N</b> .
	'e Sciences ————————————————————————————————————	
cartridge id :	MyCartridge	]
fov count :	280 fov (high)	*
email :	user@nanostring.com	]
samples :	000000000000	*
1	cancel back done	

Figure 75: The 'Cartridge information' screen.

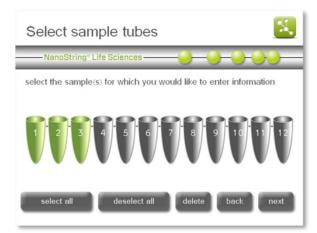


Figure 76: The 'Select sample tubes' screen.



11. Select the tubes for which sample information will be entered by touching the appropriate tube on the screen. To select all tubes and enter the same information for all, press the select all button. Selected samples will be highlighted in green. Press next to proceed. The 'Sample tubes' screen will appear (Figure 77).

IMPORTANT: When multiple samples are selected, the information entered will be applied to all selected samples (in this case, Sample tubes 1-3, as the top of the screen indicates).

- 12. On the 'Sample tubes' screen, the available sample definition fields are displayed. Select each button or box to open a keyboard screen to enter data. Enter the following information for each field and press **enter** to return to the sample tubes screen:
  - a. In the **sample ID** data field, enter a unique sample ID (if a single tube is selected).
  - **b.** In the **owner ID** data field, enter the owner ID.
  - **c.** In the **comments** data field, enter useful comments.
  - d. Select the library RLF data field. The 'Select Reporter Library File' screen will appear (Figure 78).
  - e. Select the RLF to use with this sample and press enter. The RLF contains the information necessary to link the digital color-coded reporter signals to their target identities.

**IMPORTANT:** Selecting the wrong RLF during CDF creation will result in incorrect Reporter Code Counts.

13. After all information for selected fields are completed, press done. This sample information will be output with the RCC data and can be used for parsing or annotating data.

The 'Cartridge information' screen will appear with the defined samples displayed in blue (**Figure 79**).

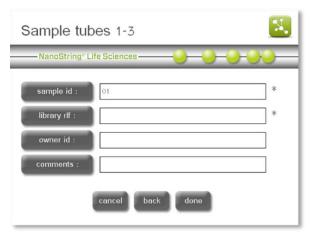


Figure 77: The 'Sample tubes' screen.



Figure 78: The 'Select Reporter Library File' screen.

Cartridge int	formation	<b>N</b> ,
NanoString® Life	e Sciences	<u> </u>
cartridge id :	MyCartridge	]
fov count :	280 fov (high)	*
email :	user@nanostring.com	
samples :	000000000000	*
	cancel back done	

Figure 79: The 'Cartridge information' screen.

If all samples for the selected cartridge are defined appropriately, press **done**. To define more samples for the cartridge, press the **samples** button and continue to enter information as described in **Steps 9-11** until all desired tubes in the cartridge have been defined. When all samples for the cartridge are defined, press **done**.

The 'Select CDF Destination' screen will appear (Figure 80).

- 14. Select an existing folder or create a new folder in which to save the CDF. Remember: navigate the folder structure by touching folders twice, similar to double-clicking with a mouse. Press next.
- 15. The 'Enter new CDF name' screen will appear (Figure 81).
- **16.** Enter the CDF name using the screen's keyboard and press **enter**.



Figure 80: The 'Select CDF Destination' screen.

Enter new cdf name cancel enter	
NanoString® Life Sciences	
Test CDF	٦
A S D F G H J K L	
x clear alt del + -	

Figure 81: The 'Enter new CDF name' screen.

If the CDF name already exists, an error will display and the instrument will ask if it should overwrite the existing file. Select no to change the name of the CDF.

A confirmation message will appear after successfully naming the CDF (Figure 82).

17. From the Main Menu, it is now possible to select start counting, using the CDF that was just created and saved (see the next section, *Initiating a Run*).



Figure 82: The new CDF name confirmation screen.



# **Initiating a Run**

1. From the Main Menu (Figure 83) select start counting.

The 'Select Stage Position' screen will appear (Figure 84).

**NOTE:** The **exit** button will only appear if the user has the nCounter Dx Analysis System with FLEX configuration (see *Selecting the Instrument Mode*). It is replaced with the **system reset** button on instruments without this configuration.

**NOTE:** The administration button will only appear if the user has the Enterprise Package enabled and is logged in as an administrator (see the *Enterprise Package* section).

2. Select the cartridge position for which cartridge information will be entered by touching that cartridge position on the screen.

The selected cartridge will appear in green. If the wrong cartridge is selected, touch the correct position and the active cartridge will display in the new position. Press **next**.

Main Menu	
NanoString® Life Sciences	-0-0-0-00-
	start counting
	create CDF
	upload files
	download data
administration	maintenance
logout	exit

Figure 83: The Main Menu.

Sele <sub>Nar</sub>		tage <sup>g® Life</sup>			n ————————————————————————————————————	K) 
	2	<b>3</b>	4		6 	select the stage position for which you would like to enter information
Position	ı #1, En	npty	са	ncel	next	done

Figure 84: The 'Select Stage Position' screen.

3. The 'Select Cartridge Definition Mode' screen will appear (Figure 85).

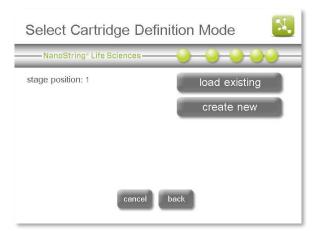


Figure 85: The 'Select Cartridge Definition Mode' screen.



 If a Cartridge Definition File (CDF) was already created and uploaded/saved to the system, press load existing. Otherwise, create a new CDF by pressing create new (see the previous section on *Cartridge Definition Files* for instructions to create a new CDF.

After pressing **load existing**, the 'Select CDF' screen will appear (Figure 86).

Select CDF
NanoString" Life Sciences
Fov-Max.cdf In4 golden CDF.cdf test cdf 2.cdf
cancel back modify next

Figure 86: The 'Select CDF' screen.

 Select the CDF to be used and press next. The 'Cartridge information' screen will appear (Figure 87).

**NOTE**: If a cartridge ID or an email address was already entered into the CDF template, then the following screens and steps may not apply to the workflow. **Skip to Step 9.** 

- To change any of the information specified for the cartridge, press the appropriate fields on the screen and follow the instructions as specified in Steps 2-11 of Creating a CDF on the Digital Analyzer. Press done.
- 7. The 'Select Stage Position' screen will appear (Figure 88).
- For additional cartridges, press **next** to repeat Steps 2-7 above until all cartridges for the run are defined.
- 9. Press done.

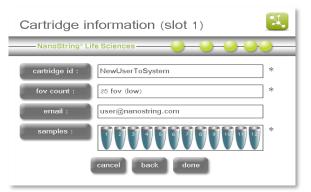


Figure 87: The 'Cartridge Information' screen, displaying details for slot 1.

Sele	ct S	tage	Po	sitio	n	X
—— Na	noStrin	g® Life	Scienc	es ——	-0	$\rightarrow$
<b>1</b>	2 <u> <u> </u> </u>	<b>3</b>	4	5	6	select the stage position for which you would like to enter information
Position	n #1, Ca	ırtridge		ewUser ncel	ToSyste	done

Figure 88: The 'Select Stage Position' screen.



Figure 89: Proper orientation of cartridges when placing them into the Digital Analyzer. Close the magnetic clips gently and be sure to push down on the metal plate above the cartridge after the lid is closed to ensure the cartridge lays flat.

- 10. Place cartridges into the stage position corresponding to the data entered in **Steps 2-7** above. Use the orientation shown in **Figure 89**. Be sure that each cartridge is seated flat in the slot. Close the magnetic clips gently.
- 11. Shut the instrument door. On the 'Initiate Imaging' screen, press **start**.

The 'Counting Cartridge ID' screen will appear (**Figure 90**). Once imaging has begun, the door will remain locked until the system is paused or the run is complete.

Once imaging begins, the 'Counting Cartridge ID' screen will display the following information:

- The cartridge ID for the active cartridge (the cartridge currently being scanned)
- Cartridge scan status/progress
  - Blue cartridge scan completed and/or in progress
  - Green cartridge yet to be scanned during the run
  - Clear (white) cartridge position for which no data has been defined
- Real-time data on the status of data collection
  - current time the current time of day as defined in the system setup utility
  - time left (#) the approximate amount of time to complete the active cartridge
  - time left (all) the amount of time to complete all cartridges
  - finish time the time of day the run will be finished (current time + total run time)

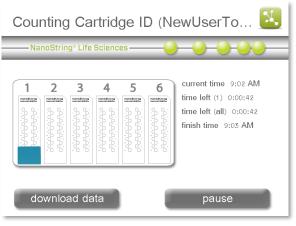


Figure 90: The 'Counting Cartridge ID' screen with information on the run in progress.

 If the Enterprise Package has been configured to require user login (see the *Enterprise Package* section), then the screen will dim and lock automatically after a run begins (Figure 91).

Press the light grey **unlock** button to log in. The screen will lighten (**Figure 92**), and the user will be able to download data (while the run continues) or pause the run.

The screen will lock again automatically if a timeout period was established. Otherwise, press the lock screen button to engage the lock manually.

13. The screen image in **Figure 93** provides an example of a completed run.

Button options during and after a run:

- download data Press download data to access data that has already been collected while the Digital Analyzer is still running or after it has finished. For instructions on downloading files, see Completing a Run.
- **pause** Press **pause** during the run to pause the run, to add additional cartridges, or to abort the run. See Pausing a Run below.

 AunoString\* Life Sciences

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Figure 91: The 'Counting Cartridge ID' screen when it is locked.

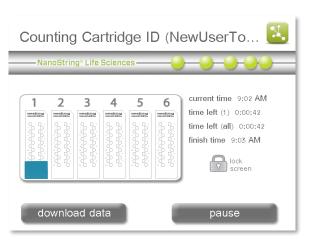


Figure 92: The 'Counting Cartridge ID' screen when it is unlocked, with the **lock screen** button visible.



• **finish** – Press **finished** when the run has completed. Pressing this button will return the application to the Main Menu.

— Na	noStrin	g® Life	Scienc	es ——		
1	2	<b>3</b> 000000000000000000000000000000000000	4	5 	6 0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,	current time 2:35 PM time left (1) 0:00:00 time left (all) 0:00:00 finish time 2:35 PM
do	ownloa	ad da	ıta			finished

Figure 93: The 'Counting Cartridge ID' screen with completed run information. If the Digital Analyzer is equipped with a Dx configuration, there will be a second button with the option to **exit to Dx** (e.g., the next user may desire to perform a run in Dx mode).



### **Pausing a Run**

From the 'Counting Cartridge' screen, runs can be paused to perform any of several actions:

- **add cartridge** A cartridge can be added if, for example, the device is in use but the Prep Station has recently finished processing samples.
- **abort cartridge** A single cartridge can be aborted; cartridges may be re-imaged.
- **abort run** The entire run can be aborted; cartridges may be re-imaged, and data from cartridges that have already been imaged will be saved.
- **upload files** New CDFs or RLFs can be uploaded if, for example, incorrect files were originally assigned when the run was initiated.

**NOTE:** The **pause** button is present while the run is still in progress. Once the run is complete, the **pause** button will be replaced by the **finished** button.

#### Add a New Cartridge

 On the 'Counting Cartridge ID' screen (Figure 90 or Figure 92), press pause.

The 'Imaging Paused' screen will appear after the imaging device halts (**Figure 94**).

- 2. Press add cartridge.
- After completing the 'add cartridge' workflow (identical to Steps 2-7 for defining a new cartridge in the 'start counting' workflow), press resume run to continue processing.

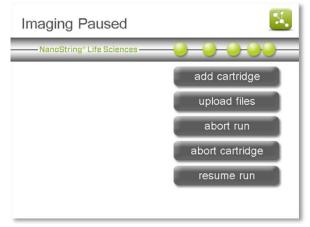


Figure 94: The 'Imaging Paused' screen.



When a run is aborted, data on cartridges and flow cells that have completed imaging are saved and available for download.

 On the 'Counting Cartridge ID' screen (Figure 90 or Figure 92), press pause.

The 'Imaging Paused' screen will appear (Figure 94).

2. To abort the run in its entirety, press abort run.

The 'Abort Run' confirmation screen will appear (Figure 95).



Figure 95: The 'Abort Run' confirmation screen.



#### 3. Press yes to abort the run.

**NOTE**: If the run is aborted but cartridges will be re-imaged, set up a new run with the same cartridges following the instructions outlined above in *Initiating a Run*.

# Abort a Cartridge

When a single cartridge is aborted, data on cartridges and flow cells that have completed imaging are saved and will be available for download.

 In the 'Counting Cartridge ID' screen (Figure 90 or Figure 92), press pause.

The 'Imaging Paused' screen will appear (Figure 94).

2. To abort the cartridge, but not the entire run, press **abort cartridge**.

The 'Abort Cartridge' confirmation screen will appear (**Figure 96**).

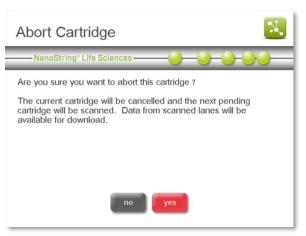


Figure 96: The 'Abort Cartridge' confirmation screen.

3. Press yes to abort the cartridge.

**NOTE:** If the cartridge is aborted but will be re-imaged, set up a new run with the same cartridges following the instructions outlined above in *Initiating a Run*.

# Upload Files

The Digital Analyzer can be paused in order to upload a new CDF or RLF. If the Enterprise Package has been enabled (see the *Enterprise Package* section), it may be configured to require that users log in before pausing the instrument.

 On the 'Counting Cartridge ID' screen (Figure 90 or Figure 92) press pause.

The 'Imaging Paused' screen will appear (Figure 94).

2. To upload new CDFs or RLFs, press upload files.

The 'Upload Files' screen will appear (Figure 97).

**3.** Follow the instructions to upload files located in the *Cartridge Definition Files* section.

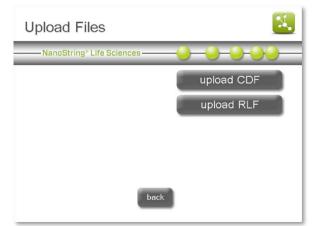


Figure 97: The 'Upload Files' screen.



# **Completing a Run**

When the data collection has completed, the stage will move into position for the door to be opened and the cartridges removed.

A Reporter Code Count (RCC) file is created for each flow cell in the cartridge. RCC files are automatically named by the Digital Analyzer using the following convention:

YYYYMMDD\_cartridgeID\_Lane Number.rcc

The output data is grouped by cartridge into a zipped folder that contains up to 12 RCC files. These zipped folders are stored on the Digital Analyzer after a run and may be retrieved in one of four ways. Directions for each method are provided in the sections that follow.

- Transfer via USB flash drive
- Transfer via email
- SSH transfer to a personal computer
- FTP transfer to a personal computer

#### **Download RCC files via USB Flashdrive**

1. From the Main Menu (Figure 98), press download data.

The 'Select Data to Download' screen will appear (Figure 99).

2. Select data for download to the RCCData folder on a USB flash drive. All data that has been processed and is available is displayed in this screen until it has been downloaded once. After the initial download, data will be moved to the 'Older Data' page.

**NOTE:** If the Enterprise Package has been enabled, it may be configured to prevent transferring files to the 'Older Data Figure 98: The Main Menu. Folder.' See the Enterprise Package section for more information.

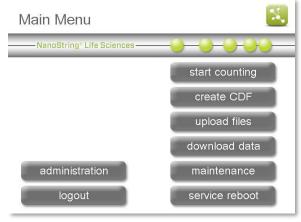
3. To select a file, touch the file name on the screen. One or more files may be selected.

Selections will turn blue.

4. To de-select a file, touch it on the screen again.

The deselected files turn from blue to white.

5. After selecting the file(s) to download, press download data.



elect Data to Downl	oad
-NanoString® Life Sciences	
20130300_arc1_RCC.ZIP 20130300_arc2_RCC.ZIP	
∟ □ older data	

Figure 99: The 'Select Data to Download' screen.



# Selecting "Older Data" Files

If data are difficult to find in the top-level window, they may be located in the 'Older Data' section.

1. In the 'Select Data to Download' screen, select the 'older data' option box (located below the file selection area; **Figure 99**).

The 'Select Older Data to Download' screen will appear, displaying all 'older data' files available for download (**Figure 100**)

2. Repeat **Steps 3-5** of the previous section to select and download older data.

Select Older Data to Download

Figure 100: The 'Select Older Data to Download' screen.

Older data is saved in dated folders and is

identified by the format **YYYYMMDD**. All data from one day may be downloaded at once. To download specific files, open the dated folder by touching it twice and select only the cartridge of interest. To go back to the top- level directory, press **(RecycleBin)** twice. Files are not removed from the Recycle Bin unless manually removed.

# **Download RCC Files via E-mail**

If Digital Analyzer networking and email have been configured via the maintenance screens and a valid email address was entered into a CDF template or during setup of a run, then RCC data from that cartridge will be delivered by email. A copy of the data will be saved in the 'Older Data' folder on the Digital Analyzer once the data have been emailed.

**NOTE:** If the Enterprise Package has been enabled, it may be configured to prevent email delivery or transferring files to the 'Older Data Folder.' See the *Enterprise Package* section for more information.

Be aware that not all institutions allow ZIP (\*.zip) files to be emailed. If having trouble receiving data, please contact the institution's IT department and verify that ZIP files are permitted.

# **Download RCC Files via SSH**

If SSH rather than FTP is preferred to transfer data files, this can be configured on the Digital Analyzer by NanoString personnel. Contact NanoString at <a href="mailto:support@nanostring.com">support@nanostring.com</a> to have SSH configured and enabled on the Digital Analyzer.



#### **Download RCC Files via FTP**

Acquire the IP address and FTP password prior to completing the following instructions to transfer data from the Digital Analyzer to a personal computer.

Go to the Maintenance Menu (**Figure 140**) on the Digital Analyzer and press system info to open the 'System Information' screen (**Figure 101**). The instrument's IP address will be located in the upper right corner.

If the instrument FTP settings have been configured, RCC files may be transferred directly to a personal computer from the Digital Analyzer. There are many FTP applications available, any of which should work for transferring data between the Digital Analyzer and a personal computer.

System	Information	<b>X</b>
NanoStrir	ig® Life Sciences ———	
Software Version	: 4.0.0.2	IP Address: 10.0.0.117
Instrument Name	e: Demo01	MAC Address: 00-50-56-A9-10-4B
Current Date:	Mon, Jul 6, 2015	Login Enabled: true
Current Time:	9:48:53 AM	Auto Logout: 2 minutes
RLF Upload:	Both USB and FTP	Use RCC older data: yes
CDF Upload:	Both USB and FTP	Disk Cleanup Enabled: true
RCC Download:	USB, FTP and Email	Barcode scanner active: yes
		Barcode tracked: log and RCC
	ok	

Figure 101: The 'System Information' screen.

1. On a Windows computer, open Windows Explorer or File Explorer. On a Macintosh computer, open Finder and select **Connect to Server...** from the Go drop-down menu. Once the file manager is open, type **FTP://(IP\_address\_here**) as shown in **Figure 102**. Press **Enter**.

I 🔁 🔟 🖛 I	This PC -	
File Computer View		~ <b>0</b>
🕒 🄄 🕆 🎼 ftp://10.0.0.117/	✓ ♂ Search This PC	ρ Connect to Server
😤 Favorites 🍋 Box Sync	A Folders (6)	Server Address:
Downloads     Recent places	Desktop	ftp://10.0.0.117 + O~
Desktop	Documents	Favorite Servers:
🌉 This PC		
Lesktop	Downloads	
Downloads Marcom Music	Music	? Remove Browse Connect
<ul> <li>Pictures</li> <li>Videos</li> </ul>	Pictures	
Local Disk (C:) 17 items	~	

Figure 102: Enter the Digital Analyzer's IP address in File Explorer (left) or Finder (right).



2. The FTP log on window will appear (Figure 103).

Log On As	
Ether the server does not allow anonymous logins or the e-mail address was not accepted.         FTP server:       10.0.0.117         User name:       technician         Password:       •         After you log on, you can add this server to your Favorites and return to it easily.         Image: TP does not encrypt or encode passwords or data before sending them to the server. To protect the security of your passwords and data, use WebDAV instead.	Enter your name and password for the server "10.0.117". Connect as: Guest Registered User Name: technician Password:
Log on anonymously  Save password  Log On  Cancel	Remember this password in my keychain     Cancel     Connect

Figure 103: The FTP log on window using File Explorer (left) or Finder (right).

3. Enter 'technician' as the FTP user name, enter the password as defined in the maintenance menu, and then click **Log On** or **Connect**.

The contents of the instrument server will appear (Figure 104).

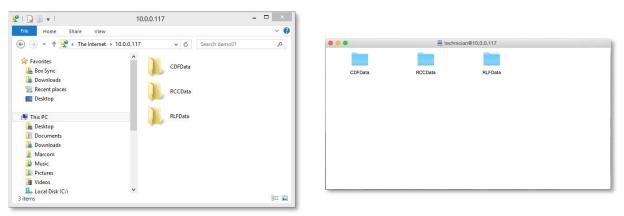


Figure 104: The FTP log on window using File Explorer (left) or Finder (right).

4. Open the RCCData folder. The RCCData folder will open and display the available files (Figure 105).

🕞 🗓 🖛 I 🛛 🖡	RCCData -					
le Home Share View		~ 🔞				
) 🌛 👻 🕆 퉬 « 10.0.0.117 🕨 RLFData	♥ 🖒 Search RCCData	م		RCCData		
Favorites	20150427_fnord_RCC.ZIP	^			BBBB	ष्ष
🔢 Recent places	20150427_lindy2_RCC.ZIP			© www.		
Desktop						
This PC	20150427_lindy3_RCC.ZIP		201	150427_fnord_RCC.ZIP	P	
This PC	20150427_lindy3_RCC.ZIP		201 Name	150427_fnord_RCC.ZIP	P	Kind
This PC				150427_fnord_RCC.ZIP		
This PC	20150427_lindy3_RCC.ZIP		Name	Date Modified	Size	ZIP archive
This PC			Name           1         20150427_fnord_RCC.ZIP           1         20150427_lindy2_RCC.ZIP           1         20150427_lindy3_RCC.ZIP	<ul> <li>Date Modified</li> <li>Apr 27, 2015, 2:30 PM Apr 27, 2015, 4:06 PM Apr 27, 2015, 4:13 PM</li> </ul>	Size 498 bytes 1 KB 1 KB	ZIP archive ZIP archive ZIP archive
This PC Desktop Documents Downloads Marcom			Name           1:         20150427, Inord, RCC.2IP           1:         20150427, Inordy, RCC.2IP           1:         20150427, Inordy, RCC.2IP           1:         20150428, Inordy, RCC.2IP	<ul> <li>Date Modified</li> <li>Apr 27, 2015, 2:30 PM</li> <li>Apr 27, 2015, 4:08 PM</li> <li>Apr 27, 2015, 4:03 PM</li> <li>Apr 28, 2015, 4:03 PM</li> </ul>	Size 498 bytes 1 KB 1 KB 499 bytes	ZIP archive ZIP archive ZIP archive ZIP archive
This PC Decktop Documents Documents Warcom Marcom Marcom Masic	20150428_lindy_RCC.ZIP		Name 1 20150427, fnord, RCC.2IP 1 20150427, fnord, RCC.2IP 1 20150427, lindy2, RCC.2IP 1 20150428, lindy3, RCC.2IP 1 20150428, lindy3, RCC.2IP 2 20150428, lindy3650548m01, RCC.2IP	<ul> <li>Dete Modified</li> <li>Apr 27, 2015, 2:30 PM</li> <li>Apr 27, 2015, 4:08 PM</li> <li>Apr 27, 2015, 4:13 PM</li> <li>Apr 28, 2015, 4:03 PM</li> </ul>	Size 498 bytes 1 KB 1 KB 499 bytes 527 bytes	ZIP archive ZIP archive ZIP archive ZIP archive ZIP archive
This PC Desktop Documents Ocuments Marcom Music Pictures	20150428_lindy_RCC.ZIP 20150428_NewUserToSystem01_RC		Name         *           1         20150427, fnord, RCC.2/P           1         20150427, indra, RCC.2/P           1         20150428, indra, RCC.2/P	150427_fmird_RCC.2IP <ul></ul>	Size 498 bytes 1 KB 1 KB 499 bytes 527 bytes 528 bytes	ZIP archive ZIP archive ZIP archive ZIP archive ZIP archive ZIP archive
This PC	20150428_lindy_RCC.ZIP 20150428_NewUserToSystem01_RC	Ţ	Name 1 20150427, fnord, RCC.2IP 1 20150427, fnord, RCC.2IP 1 20150427, lindy2, RCC.2IP 1 20150428, lindy3, RCC.2IP 1 20150428, lindy3, RCC.2IP 2 20150428, lindy3650548m01, RCC.2IP	<ul> <li>Dete Modified</li> <li>Apr 27, 2015, 2:30 PM</li> <li>Apr 27, 2015, 4:08 PM</li> <li>Apr 27, 2015, 4:13 PM</li> <li>Apr 28, 2015, 4:03 PM</li> </ul>	Size 498 bytes 1 KB 1 KB 499 bytes 527 bytes	ZIP archive ZIP archive ZIP archive ZIP archive ZIP archive

Figure 105: The contents of the 'RCCData' folder when using File Explorer (left) or Finder (right).



5. Copy and paste files from the RCCData folder to the personal computer.

**NOTE:** RCC files can be downloaded from the Digital Analyzer FTP, but these files cannot be uploaded to the instrument.

# **Technical Support & Maintenance**

# **Technical Support**

Technical support is available by phone, fax, mail, or email. Be sure to include the product number and serial number in all communications.

# U.S. Contact Information: NanoString Technologies, Inc.

530 Fairview Ave N, Seattle, Washington 98109 USA

Phone: +1 888 358 NANO (+1 888 358 6266)

Fax: +1 206 378 6288

Email: support@nanostring.com

Website: www.nanostring.com



# **Prep Station Maintenance**

There are several tasks that can be accomplished through the use of the Maintenance screens on the Prep Station:

- system setup Set the date and time on the system clock and configure e-mail
- hardware Home robot, align electrodes, lubricate O-rings and run a dry run
- **software** Update Prep Station's system software when a new release occurs and view the current software information (the 'About' screen)
- troubleshoot Download log files for remote analysis at NanoString
- shutdown system System shutdown

To access the Maintenance Menu, press **maintenance** on the Main Menu (**Figure 106**).

The Maintenance Menu will appear (Figure 107).

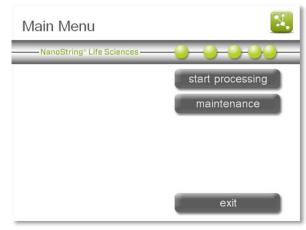


Figure 106: The Prep Station Main Menu.

Maintenance	<b>N</b>
NanoString® Life Sciences	0000
Computer Name: NA	system setup
IP Address:	hardware
MAC Address: ## - ## - ## - ## - ## - ##	software
*** - *** - *** - *** - ***	troubleshoot
	shutdown system
back	

Figure 107: The Prep Station Maintenance Menu.



#### System Setup

The System Setup Menu allows the user to set the Prep Station's date and time and configure e-mail settings. To access it, press **system setup** on the Maintenance Menu (**Figure 107**).

The System Setup Menu will appear (Figure 108).

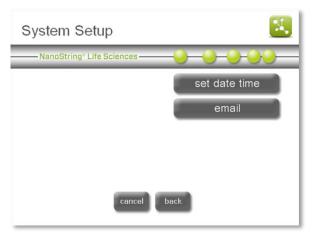


Figure 108: The System Setup Menu.

#### Setting the System Clock

Log files are created by the Prep Station and can be important for troubleshooting purposes. Setting the system clock ensures the accurate time is displayed on the screen and in the log files. The system clock must be manually set during the initial system setup and for adjustments such as Daylight-Saving Time.

#### Press set date and time.

The 'Set Date and Time' screen will appear (**Figure 109**).

# Set Date and Time NanoString\* Life Sciences Date (mm/dd/yyyy) / / 7 9 4 5 1 2 10/28/2011 16:33:24 0 delete save

Figure 109: The 'Set Date and Time' screen.

**IMPORTANT:** If operating the nCounter Dx Analysis System with FLEX configuration, a user can only change the system date and time through the Web Application while logged in as an administrator. See the *nCounter Dx Analysis System User Manual* (MAN-C0034) for more information.



# Email Access

Email addresses can be added, deleted, managed, and saved on the Prep Station. This function allows users to receive notifications via email. Email must be configured for the notifications to work properly.

To configure e-mail, press the **email** button on the System Setup Menu (**Figure 108**).

The 'Email configuration' screen will appear (**Figure 110**).

**NOTE:** Please contact trained IT personnel for assistance with setting up email on the Prep Station.

Email configuration	
NanoString Life Sciences	
smtp server :       *         smtp port :       25         from address :       user@nanostring.com         from security :       disabled         back       save       test         email accounts	

Figure 110: The 'Email configuration' screen.

There are several settings required to configure e-mail on the Prep Station. Use the arrows on the right to advance through all settings:

- **SMTP server:** Simple Mail Transport Protocol (SMTP) is the network protocol used to send email across the Internet. This setting must be configured correctly or outgoing email will not be sent. Enter the name of the SMTP server for outgoing email.
- **SMTP port:** Enter the name of the port used by the SMTP server.
- **from address:** Enter a valid email address. This address will be seen in the "From" line of any outgoing emails sent from the system.
- **from security:** Security is disabled by default. To enable security on the Prep Station, press from security and then enter the from password.
- from password: If security is enabled, enter the appropriate password for the email address.
- **admin address:** In addition to user emails specified when processing a run, administrators can receive email notifications as well.
- **notify admin:** Designate whether the administrator will receive email notifications upon every run completion or disable it to not send email notifications to the administrator.

**NOTE:** It is recommended that email settings be tested prior to setting up a run by selecting the test button and checking the inbox of the test email address.



Email accounts can be deleted from the Prep Station as well. To delete an email address, press the email accounts button from the 'Email configuration' screen (**Figure 110**).

The 'Email Accounts' screen will appear (Figure 111).

To delete an email account from the Prep Station, select that email address and press **delete**.

Email Accounts		
NanoString® Life Sciences		
please select the email addresses you would like to delete from the system	user@nanostring.com	
	back delete	

Figure 111: The 'Email Accounts' screen



# System Hardware

To access the System Hardware Menu, press hardware on the Maintenance Menu (Figure 107). The System Hardware Menu (Figure 112) enables the user to:

- **home robot** Homes all the motors should the robot enter an undesired state.
- align electrodes Guides the user in how to align electrodes if they become bent and no longer fit into the cartridge.
- **lubricate O-rings** Alerts the user when the O-rings need to be lubricated and guides the user in the process.
- **dry run** Performs a dry run of the system if it has been dormant for an extended time.

#### Home Robot

If the robot enters an undesirable state, pressing the **home robot** button can home all the motors. This button should not be needed under normal operation.

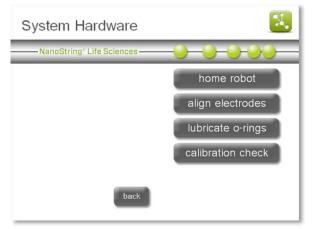


Figure 112: The System Hardware Menu.

Home Prep Station	<b></b>
NanoString® Life Sciences	
Initializing prep station	

Figure 113: The 'Home Prep Station' screen.



# Align Electrodes

From time to time, the electrodes used for stretching the reporters may become bent out of alignment and no longer fit into the cartridge. This usually occurs if the cartridge was not aligned properly when the electrode fixture was depressed causing the electrode to make contact with the cartridge and bend. The following describes a method for re-alignment of the electrodes using the system hardware interface. This method allows the electrode fixture to tilt at an angle that makes it much easier to view the electrodes during alignment.

If the electrode is so severely bent that it cannot be inserted into a cartridge, the process outlined below may need to be repeated twice - once without a cartridge present to get the electrodes into roughly the right position so that they can be inserted into a cartridge and a second time with a cartridge present for fine tuning the placement of electrodes in the cartridge well.

**NOTE:** The Prep Station checks electrode function during the validation steps of the process before any samples are processed. If it detects problems with an electrode, the processing will halt and wait for user intervention. The screens described below will then appear, allowing the user to fix the bent electrodes.

 Select align electrodes on the System Hardware Menu (Figure 112).

The 'Align Electrodes – STEP 1' screen will appear (**Figure 114**).

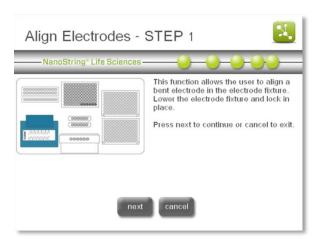


Figure 114: The 'Align Electrodes - STEP 1' screen.

2. If the electrode is severely bent to the point where a cartridge cannot be inserted, first close the electrode fixture without a cartridge in it and adjust the position of the electrode such that it is positioned similarly to the other electrodes.

If the electrode is only slightly bent and a cartridge can be inserted by carefully maneuvering the bent electrode, then insert a cartridge before closing the electrode fixture. Press **next**.

The 'Align Electrodes Menu – STEP 2' screen will appear (Figure 115).

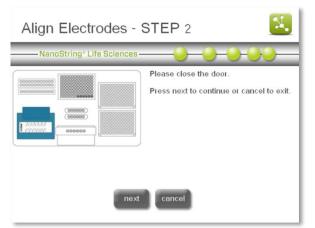


Figure 115: The 'Align Electrodes - STEP 2' screen.

3. Close the door and select **next**. The electrode fixture will tilt towards the operator for easier access to the electrodes.

Two screens will appear in succession, ending on Step 3 (Figure 115).

Align Electrodes - STEP 3		
NanoString* Life Sciences -		
Need Help? Contact NanoString technical support: support@nanostring.com 1-883-358-6266	Open the door and align the electrodes. When the electrodes have been aligned, press next to continue.	

Figure 116: The 'Align Electrodes - STEP 3' screen.

 Using a pair of tweezers or fine tip pliers, bend the electrodes into alignment. Try to align the tip of the electrode to the center and bottom of the cartridge well. Press **next** when done.

The 'Align Electrodes Menu – STEP 4' screen will appear (**Figure 117**).

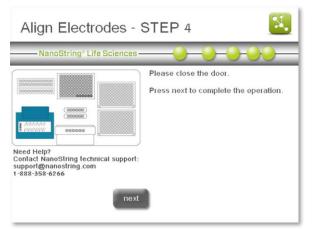


Figure 117: The 'Align Electrodes - STEP 4' screen.

5. Close the door and the electrode fixture will tilt back to its home position. Press **next**.

Two screens will appear in succession, ending on Step 5 (Figure 118).

6. Press **finish** to return to the Maintenance Menu.

Align Electrodes - STEP 5		
	Electrode alignment has completed. Press finish to return to system hardware screen.	
finish		

Figure 118: The 'Align Electrodes - STEP 5' screen.



## Lubricate O-Rings

**CAUTION:** When conducting maintenance activities, always use appropriate safety precautions including wearing safety glasses and gloves.

**ADVERTISSEMENT:** Pendant les tâches de maintenance, toujours mettre en œuvre les mesures de précaution appropriées, notamment le port de gants et de lunettes de sécurité.

The O-rings on the pipetting nozzles in the Prep Station are present to ensure a good seal with the pipette tips, thus ensuring accurate liquid volume control. O-rings are the small black rings found on the lower end of the nozzle. These O-rings must be lubricated once a month to maintain a good seal.

The Prep Station Maintenance Menu has an interface to make O-ring lubrication easier. Following the user interface brings the pipetting head to the front of the deck for access during lubrication.

Required materials:

- Silicone Grease (light grey, transparent grease in a plastic container)
- Lint-free Paper (e.g., Kimwipe)
- Rubber Gloves
- Paper clip

An alert will appear on the main screen and maintenance screen when it is time to lubricate the O-rings (Figure 119).



Figure 119: The 'lubricate O-rings' maintenance alert screens.

NOTE: The Prep Station will continue to function normally even though the yellow alert icon is present.



 Select lubricate O-rings on the Main Menu or on the System Hardware Menu (Figure 110).

A series of 'Lubricate O-rings' maintenance screens will appear (**Figure 120**).

2. Follow the instructions as directed on the screen (Figure 121).



Figure 120: The 'Lubricate O-rings – STEP 1' screen.

3. To lubricate the O-rings, first use a twisted Kim Wipe or lens cloth to "floss" between the nozzles and remove any grease from the white tip ejector block above the nozzles. If necessary, pull the tip ejector block down to thoroughly clean the end of it. Next, take a small amount of silicone grease and rub the tip around the O-ring using a gloved hand as shown (Figure 122).

**IMPORTANT:** DO NOT USE ANYTHING OTHER THAN THE SUPPLIED SILICONE GREASE ON O-RINGS.

**IMPORTANT:** Excessive grease may cause equipment malfunction. To test before running samples, perform the Dry Run protocol (described below).



Figure 121: The 'Lubricate O-rings – STEP 2' screen.

4. Continue following the instructions in each step of the workflow. Using lint-free paper, thoroughly wipe off any excess silicone grease from the nozzles and tip ejectors. Be careful to not get grease into the nozzle itself. If grease does get in the nozzle, un-bend a paperclip and very carefully use the wire to scrape the grease back out.

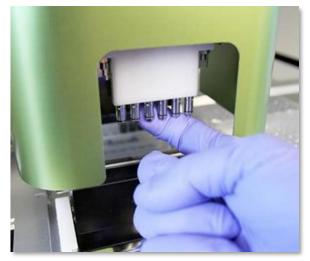


Figure 122: Use a finger to apply a small amount of NanoString silicone grease to the O-rings

5. At the end of the workflow (Figure 123), press finish to return to the Maintenance Menu.

Completing the Lubricate O-rings process will reset the internal timer and remove the yellow alert icon if it is present.



Figure 123: The 'Lubricate O-rings – STEP 5' screen.



# nCounter Prep Station - Maintenance Dry Run

**NOTE:** When conducting maintenance activities, always use appropriate safety precautions including wearing safety glasses and gloves.

If the Prep Station has been idle for more than a month, it is highly recommended to perform a **dry run** on this equipment, preferably several days in advance of the planned run. The purpose of a dry run is to lubricate hardware which would ordinarily be lubricated by routine use. This will help ensure that moving parts do not get stuck while processing precious samples, and will thus reduce the risk of losing precious samples.

To perform the dry run, you will need the following "blank" reagent components. It is appropriate to employ used reagents for this protocol.

- Full set of used pipette tips
- 2x blank prep plates (opened foil on the top, but no green label on the side)
- 2x tip sheaths
- 3x blank 12-sample strip tubes
- Blank cartridge
- 1. After locating the blank reagents, turn on the Prep Station and wait for it to boot up.

**NOTE:** If the instrument has been idle, the "lubricate O-rings" message will most likely be present on the Home screen. It is recommended to clean and relubricate the O-rings prior to the dry run.

- 2. Open the door and load the blank reagents onto the Prep Station.
- 3. Close the door, and from the Main Screen choose the "start processing" function.
- Once you get to the sample designation screen, process all twelve 'blank' samples, and make sure to check the box to launch the Legacy Protocol (Figure 124).

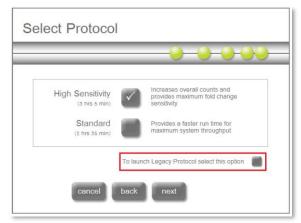


Figure 124: 'Select Protocol' screen



5. Approximately 10 minutes after initiating the dry run, you will receive a 'bent electrode' error message (**Figure 125**). This is expected as there is no liquid in the blank cartridge. To continue with the run, choose 'IGNORE' and the Prep Station will continue running the rest of the protocol.

**NOTE**: It is recommended that you stay with the Prep Station during the dry run, to correct any issues that may occur throughout. If you do not stay with the instrument the entire time, plan to return approximately 10 minutes after initiating the dry run to press IGNORE on the 'bent electrode' error, or the protocol will not finish.

- 6. When the dry run is complete, please save the discarded tips by loading them back into the tip rack, as well as the empty plates, tubes, and tip sheaths. These can be reused in any future dry runs.
- 7. Proceed with your experimental run as planned.

If you encounter any issues with the dry run, please reboot the instrument and attempt a second dry run. If the second dry run does not complete, please download the Prep Station log files and send them to support@nanostring.com.

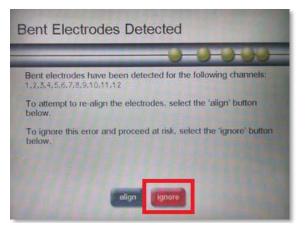


Figure 125: 'Bent Electrodes Detected' screen



## System Software

To access the System Software Menu, press software on the Maintenance Menu (**Figure 107**). The System Software Menu (**Figure 126**) can be used to update the Prep Station's system software when a new release occurs and to view the current software version information.

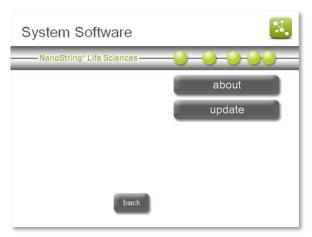


Figure 126: The System Software Menu.

#### About

Should assistance with the Prep Station be required, please determine the system software version prior to calling NanoString. This information can be found in the 'About' section on the System Software Menu (**Figure 127**).



Figure 127: The 'About' screen.

# Update

Periodically, NanoString will release new version of software for the instruments. Users can download the latest version of the software from the NanoString Technologies website and will be required to transfer the update files to a USB flash drive prior to updating the software. To update the software, insert the USB flash drive into the USB port on the side of the touchscreen and select the **update** button. The system will automatically perform the software update.



#### Troubleshoot

In the event of technical difficulties, NanoString support staff will ask the user to download and email log files to them for diagnosis of the issue. To access the Troubleshoot Menu, press **troubleshoot** on the Maintenance Menu (**Figure 107**). The Troubleshoot Menu (**Figure 128**) is used to gain access to the screen where files can be selected for download. Users also have the option to run specialized procedures that may assist in recovering failed runs.

Troubleshoot	<b>1</b>
NanoString® Life Sciences	-0-0-0-0-
	download log files
	start binding
	start immobilizing
cancel	

Figure 128: The Troubleshoot Menu.

#### Download Log Files

Access log files by pressing **download log files** on the Troubleshoot Menu (**Figure 128**). Log files are labeled with the date of each run. Multiple folders may be selected for download by touching them (**Figure 129**). Selected folders will be highlighted in blue.

- Insert a USB flash drive into the Prep Station's front USB port.
- When the correct folders have been selected, press download data to transfer them to the USB flash drive.

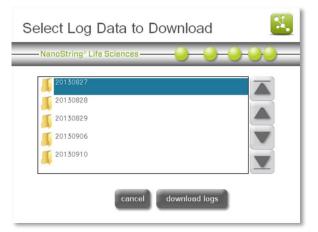


Figure 129: The 'Select Log Data to Download' screen.



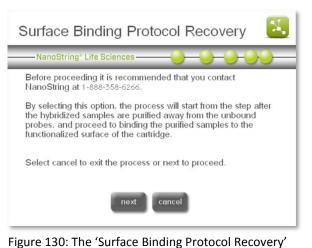
# Start Binding

The 'Start Binding' protocol can assist in running specialized procedures or in recovering failed runs. The process will start from the step after the hybridized samples are pulled away from the unbound probes and proceed to binding the purified samples to the surface of the cartridge.

**NOTE:** Always contact support@nanostring.com prior to starting binding recovery.

1. To initiate the surface binding protocol recovery workflow, press **start binding** on the Troubleshoot Menu (**Figure 128**).

The 'Surface Binding Protocol Recovery' screen will appear (Figure 130). Press next.



screen.

- Instructions will appear on the next screen (Figure 131). Follow the instructions on the touchscreen to configure the system for the surface binding protocol.
  - a. Purified samples will be dispensed, in order, in columns 7-12 of row D of the plate(s).
  - b. Place plates on the deck.
  - c. Load a new rack of tips.
  - d. Load a sample cartridge.
  - e. Place tip sheaths on the deck.
  - f. Empty the waste bin and place it on the deck.
  - g. Close the heater lid and electrode fixture.
  - h. Press next.

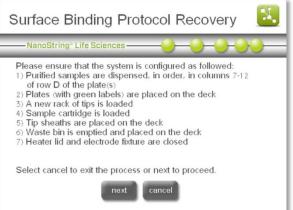


Figure 131: The 'Surface Binding Protocol Recovery' instructions.



The 'Sample Selection' screen will appear (Figure 132). After selecting the samples to be processed, press finish.

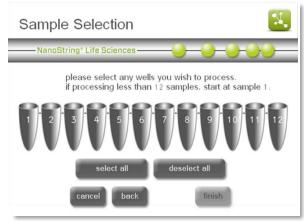


Figure 132: The 'Sample Selection' screen.

- The 'Notification Options' screen will appear (Figure 133).
- Select an email address to receive an email alert when the protocol is complete, and set the system to produce an audio alert if desired. Press next.

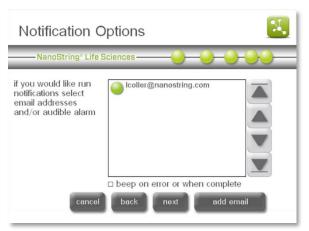


Figure 133: The 'Notification Options' screen

The 'Start Processing' screen will appear (Figure 134). Press start to initiate the protocol. Refer to *Operating the Prep Station* for further instructions.

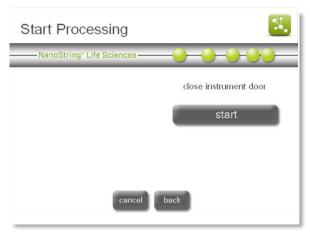


Figure 134: The 'Start Processing' screen.



## Start Immobilizing

The 'Start Immobilizing' protocol can assist in running specialized procedures or in recovering failed runs. The process will start from the step after the hybridized samples are bound to the functionalized surface of the cartridge and proceed to immobilization of the bound samples.

NOTE: Always contact support@nanostring.com prior to starting surface immobilization

 To initiate the 'Surface Immobilization Protocol Recovery' workflow, press start immobilizing on the Troubleshoot Menu (Figure 128).

The 'Surface Immobilization Protocol Recovery' screen will appear (**Figure 135**). Press **next** to proceed with the protocol.

Surface Immoblization Protocol Re 送	
NanoString" Life Sciences	
Before proceeding it is recommended that you contact NanoString at 1-888-358-6266.	
By selecting this option, the process will start after the samples have been bound to the functionalized surface of the cartridge, and proceed to complete immobilization of the bound samples.	
Select cancel to exit the process or next to proceed.	
next cancel	

Figure 135: The 'Surface Immobilization Protocol Recovery' screen.

- Instructions will appear on the next screen (Figure 136). Follow the instructions on the touchscreen to configure the system for the surface immobilization protocol.
  - a. Confirm that the surface binding and cartridge buffer exchange are completed.
  - **b.** Place plates on the deck.
  - c. Load a new rack of tips.
  - d. Load a sample cartridge.
  - e. Place tip sheaths on the deck.
  - f. Empty the waste bin and place it on the deck.
  - g. Close the heater lid and electrode fixture.
  - h. Press next.

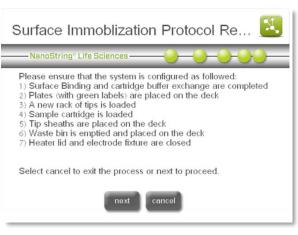


Figure 136: The 'Surface Immobilization Protocol Recovery' instructions.



 Additional instructions will appear. These instructions apply only to recovery of 12 samples (Figure 137). Press next.



Figure 137: Additional instructions for the 'Surface Immobilization Protocol Recovery' workflow.

The 'Sample Selection' screen will appear (Figure 138). After selecting the samples to be processed, press finish.

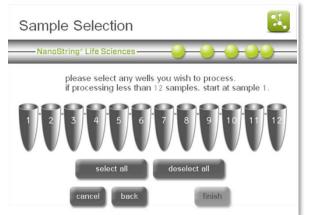


Figure 138: The 'Sample Selection' screen.

The 'Notification Options' screen will appear (Figure 139). Select an email address to receive an email alert when the protocol is complete, and set the system to produce an audio alert if desired. Press next.

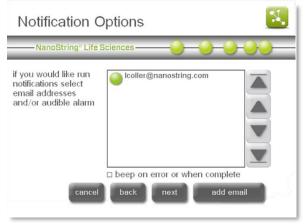


Figure 139: The 'Notification Options' screen.



The 'Start Processing' screen will appear (Figure 140). Press start to initiate the protocol.

Refer to the *Operating the Prep Station* section for further instructions.

Start Processing	<b>N</b>
NanoString® Life Sciences	9-9-9-99
	close instrument door
1	start
cancel bad	ck

Figure 140: The 'Start Processing' screen.

# Shutdown System

If the system needs to be shut down, it is preferable to use the **shutdown system** button on the Maintenance Menu (**Figure 107**) to power off the system before using the switch on the back of the Prep Station.

# **Digital Analyzer Maintenance**

There are several tasks that can be accomplished through the use of the Maintenance screens on the Digital Analyzer.

To access the Maintenance Menu, press maintenance on the Main Menu (Figure 141).

The Maintenance Menu will appear (Figure 142). The following options are available from the Maintenance menu:

- system setup Set the date and time on the system clock, configure e-mail settings, and set the FTP password.
- software Update the Digital Analyzer's system software when a new release occurs, view the current software version information, and restart the system in troubleshooting situations.
- troubleshoot Download log files for remote analysis by NanoString.
- user profile Edit the current user's profile. (This task will not appear if a user is not logged in.)
- system info View a summary of the current instrument configuration, including administration settings, software version, instrument name, date and time, IP address, and MAC address.
- *disk cleanup* View the capacity of the instrument hard drive and delete unwanted RLFs, CDFs, and RCC files.
- shutdown system Use this button before reaching Figure 142: The Digital Analyzer Maintenance Menu. around the back and flipping the power switch.

Each option is described in detail on the following pages.

Main Menu	<b>X</b>
NanoString® Life Sciences	-0-0-0-00-
	start counting
	create CDF
	upload files
	download data
administration	maintenance
logout	exit

Figure 141: The Digital Analyzer Main Menu.

Maintenance Menu	<b>1</b>
NanoString® Life Sciences	0000
system setup	system info
software	disk cleanup
troubleshoot	shutdown system
user profile	
back	



#### System Setup

The 'System Setup' screen (**Figure 143**) in the Maintenance section allows the user to set the Digital Analyzer's date and time, configure e-mail settings, and set the FTP server password.

System Setup	<b>N</b>
	-0-0-0-0-
	date time
	email
	FTP
	SSH info
cancel	back

Figure 143: The 'System Setup' screen.

## Setting the System Clock

The data files created by the Digital Analyzer are named with a date and time format. The system clock must be manually set during the initial system setup and for adjustments such as Daylight-Saving Time (**Figure 144**).

Set Date and Tim	е				М,
NanoString <sup>®</sup> Life Science	5	-0	-0	)-0	 <u> </u>
Date (mm/dd/yyyy)	7	8	9		
Time (hh:mm:ss)	4	5	6		
10/28/2011 16:33:24	1	2	3		
	0	del	ete		
sa	/e	back			

Figure 144: The 'Set Date and Time' screen.

**IMPORTANT:** If operating the nCounter Dx Analysis System with FLEX configuration, a user can only change the system date and time through the Web Application while logged in as an administrator. See the *nCounter Dx Analysis System User Manual* (MAN-C0034) for more information.



# E-mail Address

The system can send data to the user's e-mail account if this option is configured (**Figure 145**). In the system screen, the user is only setting up the outgoing, or 'from', e-mail information. The 'to' information is entered for each cartridge individually when setting up a run. RCC files will be compressed into a ZIP file and e-mailed to the account listed for each cartridge.

**NOTE:** Contact trained IT personnel for assistance when configuring email settings on the Digital Analyzer.

Email configuration	<b>N</b> .
NanoString® Life Sciences	
smtp server :	

Figure 145: The 'Email configuration' screen.

There are several settings required to configure e-mail on the Digital Analyzer. Use the arrows on the right to advance through all settings:

- **SMTP server** Simple Mail Transport Protocol (SMTP) is the network protocol used to send email across the Internet. This setting must be configured correctly or outgoing email will not be sent. Enter the name of the SMTP server for outgoing email.
- **SMTP port** Enter the name of the port used by the SMTP server.
- from address Enter a valid email address. This address will be seen in the "From" line of any outgoing emails sent from the system.
- **from security** Security is disabled by default. To enable security on the Prep Station, press from security and then enter the from password.
- from password If security is enabled, enter the appropriate password for the email address.
- **test address** After entering the above information, enter a valid email address to test the email settings.

**NOTE:** NanoString recommends testing email settings prior to setting up a run. Press the test button and confirm that a message was delivered to the inbox of the test email address.

Email accounts can be deleted from the Digital Analyzer as well. To delete an email address, press the **email accounts** button from the 'Email configuration' screen (**Figure 145**).



The 'Manage email addresses' screen will appear (Figure 146).

Email addresses associated with a user profile are not listed on this screen. Instead, use this screen to enter additional email addresses without creating a user profile. To delete an email address from the Digital Analyzer, select that email address and press **delete**.



Figure 146: The 'Manage email addresses' screen.

# FTP Setup

- To configure FTP access to the Digital Analyzer, select FTP on the 'System Setup' screen. The 'FTP configuration' screen will appear (Figure 147). The default FTP username is set to "technician." The username cannot be changed.
- 2. To set or change the password at any time, press the password button and type another password. It will overwrite the existing one.
- To disable FTP access to the Digital Analyzer, press the enabled box so that it displays "no (disabled)", and no FTP access will be allowed.

	Life Sciences	-00-
enabled :	yes (enabled)	*
username :	technician (not configurable)	*
password :	******	*

Figure 147: The 'FTP configuration' screen.

**NOTE:** Be aware that anyone with physical access to the Digital Analyzer can enable FTP or overwrite the password.

#### SSH Setup

NanoString personnel can configure the Digital Analyzer if users prefer to use SSH rather than FTP to transfer data files. Contact NanoString at support@nanostring.com to have SSH configured and enabled on the Digital Analyzer (**Figure 148**).



Figure 148: The 'SSH configuration' screen.



#### System Software

Select **software** from the Maintenance Menu (**Figure 140**) to update Digital Analyzer's system software when a new release occurs, view the current software version information, and restart the system in troubleshooting situations (**Figure 149**).

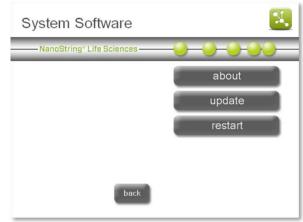


Figure 149: The 'System Software' screen.

Should assistance with the Digital Analyzer be necessary, please identify the system software version prior to calling NanoString. This information can be found in the **about** section on the System Software screen (**Figure 150**).



Figure 150: The 'About' screen.

#### Update

Periodically, NanoString will release a new version of software for the instruments. Users can download the latest version of the software from the NanoString Technologies website and will be required to transfer the update files to a USB flash drive and placed in a folder labeled "Installer" prior to updating the software. To update the software, insert the USB flash drive into the USB port on the side of the touchscreen and select the update button. The system will automatically perform the software update.

# Restart

In rare troubleshooting situations, a NanoString representative may ask the user to restart the Digital Analyzer. Ensure that the cartridge door is closed, and then select the **restart** button.



# Troubleshoot

Use the Troubleshoot Menu to download log files to send to NanoString when there is a problem that cannot be identified.

- Press troubleshoot on the Maintenance screen. The 'Troubleshoot' screen will appear (Figure 151).
- 2. Press the download log files button.

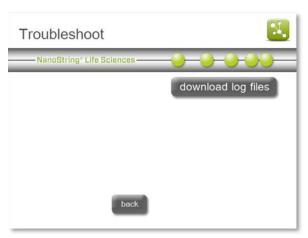


Figure 151: The 'Troubleshoot' screen.

- 3. The 'Select Log Data to Download' screen will appear (Figure 152). Insert the USB flash drive into the USB port on the right of the touchscreen.
- Navigate to the appropriate log file directory by pressing on the folder. Log files are organized by date the scan was initiated. Press the download logs button.

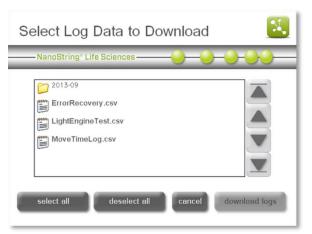


Figure 152: The 'Select Log Data to Download' screen.

5. When the files have successfully downloaded, a confirmation message will appear (Figure 153). Take the USB flash drive out of the instrument and insert it into a personal computer. Email the log files to support@nanostring.com. A member of the NanoString Technical Support team will review the log files to help determine the cause of any issues.

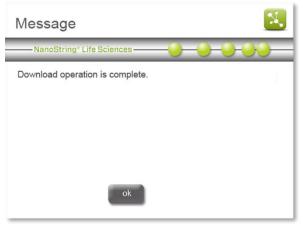


Figure 153: The 'Successful download' message screen



# **User Profile**

If the user is logged in, this option can be used to edit the information in that user's profile. See the Enterprise Package instructions in *Operating the Digital Analyzer* for more information about the details included in user profiles.

## **System Information**

The 'System Information' screen (**Figure 154**) contains key information about the configuration of the Digital Analyzer. Some of these settings can only be configured by NanoString Support or from other screens accessed from the Maintenance Menu. These include:

- Software Version
- Instrument Name
- Current Date
- Current Time
- IP Address
- MAC Address

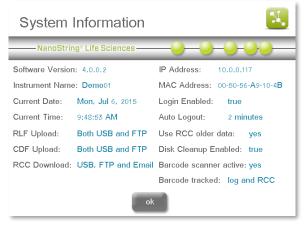


Figure 154: The 'System Information' screen.

Additional settings are configured through the Administration Menu and will only be available if the Enterprise Package is enabled. See *Operating the Digital Analyzer* for more information.

#### **Disk Cleanup**

Select **disk cleanup** from the Maintenance Menu (**Figure 142**) to check the current disk space usage (used and free), to delete RCC files, to delete CDFs, and to delete RLFs (**Figure 155**).

**NOTE:** If the Enterprise Package is enabled, it is possible to configure these settings so that files cannot be deleted through disk cleanup. See *Operating the Digital Analyzer* for more information.

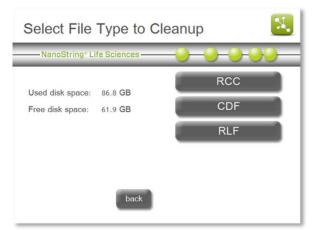


Figure 155: The 'Disk Cleanup' screen.



# Cleanup RCC Files

- To cleanup RCC files, press RCC on the 'Disk Cleanup' screen. The 'Select Files to Delete' screen will appear (Figure 156).
- 2. Select one or more files to delete and press delete.
- 3. At the prompt, confirm the files to delete by pressing **yes**.

Select Files to Delete	<b>X</b>
NanoString* Life Sciences ————————————————————————————————————	
<ul> <li>20130314</li> <li>20130502</li> <li>20130518</li> <li>20130300_arc1_RCC.ZIP</li> <li>20130300_arc2_RCC.ZIP</li> </ul>	
select all deselect all cancel bac	k delete

Figure 156: The 'Select Files to Delete' screen.

# Cleanup CDFs

- To cleanup Cartridge Definition Files, press CDF on the 'Disk Cleanup' screen. The 'Select Files to Delete' screen will appear (Figure 157).
- 2. Select one or more files to delete and press delete.
- 3. At the prompt, confirm the files to delete by pressing **yes**.

Select Files to Delete	
NanoString* Life Sciences	
LI Folder LI CDF 100 .cdf Test CDF.cdf	
select all deselect all cancel back delete	

Figure 157: The 'Select Files to Delete' screen.

# Cleanup RLFs

- To cleanup Reporter Library Files, press RLF in the 'Disk Cleanup' screen. The 'Select Files to Delete' screen will appear (Figure 158).
- 2. Select one or more files to delete and press delete.
- 3. At the prompt, confirm the files to delete by pressing **yes**.

Select Files to Delete
NanoString* Life Sciences
n6_vnTrain48.rlf n6_vRCC16.rlf
select all deselect all cancel back delete

Figure 158: The 'Select Files to Delete' screen.

#### Shutdown System

If the system needs to be shut down, it is preferable to use the **shutdown system** button on the Maintenance Menu (**Figure 140**) to power off the system before using the switch on the back of the Digital Analyzer.

**IMPORTANT:** If operating the nCounter Dx Analysis System with FLEX configuration, the system can only be shut down while the user is logged in as an administrator and operating the instrument in Diagnostics mode. See the *nCounter Dx Analysis System User Manual* (MAN-C0034) for more information.



# Cleaning

# **Prep Station**

After a completed run, remove all consumables and waste.

Periodically, clean the stage surface and waste receptacles of the Prep Station by wiping with a disinfectant followed by wiping with water or 70% ethanol. Avoid the electrode fixture. An RNase removal agent such as RNaseZap<sup>®</sup> from Ambion<sup>®</sup> may also be used.

#### Clean the exterior periodically using a diluted neutral soap followed by water.

**NOTE:** If it becomes necessary to wash the touchscreen, use a damp cloth to gently wipe the surface. Do not use excessive water or other solvents, which may damage the screen.

## **Digital Analyzer**

#### Clean the exterior periodically using a diluted neutral soap followed by water.

**NOTE:** If it becomes necessary to wash the touchscreen, use a damp cloth to gently wipe the surface. Do not use excessive water or other solvents, which may damage the screen.

# **Disposal of Electronic Equipment**



This symbol on the nCounter Analysis System is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. The presence of this marking on the product indicates that:

- The device was put on the European Market after August 13, 2005.
- The device is not to be disposed via the municipal waste collection system of any member state of the European Union.

**IMPORTANT:** For products under the requirement of WEEE directive, please contact your dealer or local NanoString office for the proper decontamination information and take back program, which will facilitate the proper collection, treatment, recovery, recycling, and safe disposal of the device.



# **Symbols and Definitions**

	Manufacturer
EC REP	Authorized Representative in the European Community
CE	CE Mark
REF	Catalogue or Reference Number
LOT	Batch code / Lot number
SN	Serial number
	Temperature range storage conditions
	Lower limit of temperature storage conditions
K	Upper limit of temperature storage conditions
	For Use by / Expiry Date
$\sim$	Date of Manufacture (DOM)

# **Regulatory Disclaimer**

For applications for which approval/clearance is not required (nCounter Dx Analysis System with *FLEX*) and research use (nCounter *MAX* Analysis System).



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