nCounter® Pro
Analysis System

nanoString

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Introduction

Product Use Limitations

The nCounter Pro Analysis System is intended for applications for which approval/clearance is not required and for research use. This 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, email 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.

Online Training at NanoString University

NanoString University is an online training platform for instructional videos and documents to accompany your NanoString instrument and assays. Create a free account at https://university.nanostring.com.

Manual Conventions

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

Text Style	Meaning
Bold	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 hyperlink 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.

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.

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.

Instrument Specifications

Test Lanes per Run	1–12
Weight	Prep Station: 216 lb/98 kg Digital Analyzer: 148 lb/67 kg
Dimensions (W x D x H)	Prep Station: 35.0 x 26.4 x 24.6 in/89 x 67 x 63 cm Digital Analyzer: 26 x 26 x 19 in/66 x 66 x 48 cm
Power Requirements	100–240 VAC, 50/60 Hz, 610 VA
Fuse	8A (100–120 VAC) or 4A (200–240 VAC)

Environmental Specifications

Indoor or Outdoor Use	Indoor
Altitude	2000 m/6561 ft
Temperature	18–28°C
Humidity	30-80% relative humidity (non-condensing)
Overvoltage Category	II
Pollution Degree	2

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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 with 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 is a piece of electrical equipment for measurement, control, and laboratory use. It 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 contact 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 codes-barres 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 est un équipement électrique pour la mesure, le contrôle et l'utilisation en laboratoire. Celle-ci utilise des modules chauffants et de la haute tension indiquées par des symboles sur le châssis. Évitez tout contact avec le module chauffant et les électrodes. L'instrument est doté d'un mécanisme de verrouillage de sécurité qui empêche l'application d'une 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.

⚠ **RISQUE ÉLECTRIQUE**   ⚠ **ATTENTION SURFACE CHAUDE**

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.
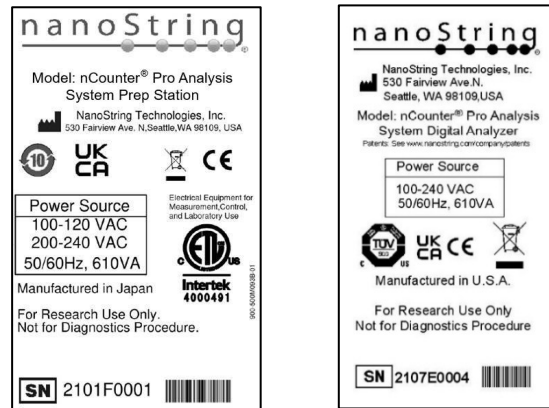

Figure 1: Serial number and compliance information for the Prep Station (left) and Digital Analyzer (right).

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**.
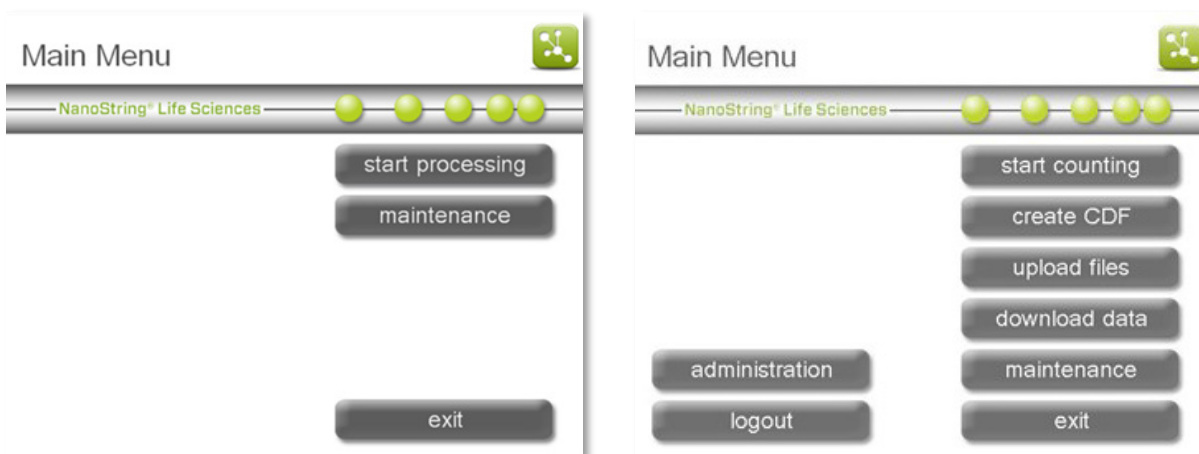

Figure 2: Main Menu on the touchscreens of the Prep Station (left) and the Digital Analyzer (right).

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 multi-channel 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 (e.g. *nCounter miRNA Assay Manual* (MAN-C0009) or *nCounter Gene Expression CodeSet RNA Hybridization Protocol* (MAN-10056)). On the Prep Station deck, hybridized samples are purified and immobilized in a Sample Cartridge for data collection (**Figure 4**).

Figure 3: The nCounter Prep Station.

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 the protocol used.
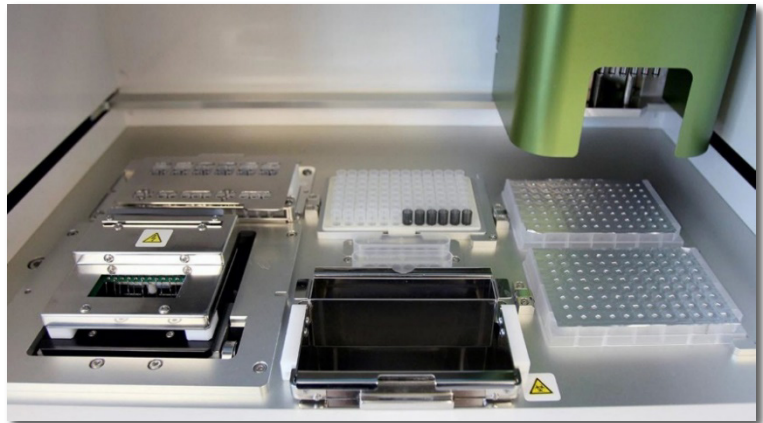

Figure 4: The Prep Station deck.

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**).

The Digital Analyzer 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 RCC files are downloaded as a zipped folder of comma separated value (CSV) files. These can be imported and analyzed using NanoString's nSolver™ Analysis Software or other data analysis and visualization software packages, such as ROSALIND cloud platform.

Figure 5: The nCounter Digital Analyzer.

Prep Station

Select Instrument Mode

After turning on the Prep Station, the first screen to appear is titled 'Select Instrument Mode' (**Figure 6**).

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.

Exit Life Sciences Mode

The user may exit Life Sciences mode by pressing the **exit** button located at the bottom of the Main Menu (**Figure 7**).

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

Figure 7: The Prep Station Main Menu in Life Sciences mode.

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.
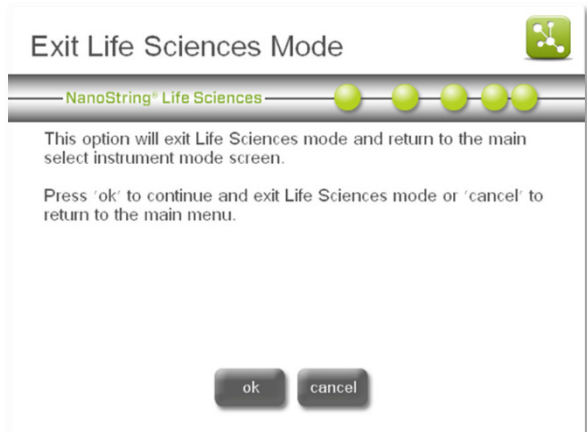
Figure 8: The Prep Station 'Exit Life Sciences Mode' confirmation screen.

Digital Analyzer

Select Instrument Mode

After turning on the Digital Analyzer, the first screen to appear is titled 'Select Instrument Mode' (**Figure 9**). 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).

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 9: The Digital Analyzer 'Select Instrument Mode' screen.
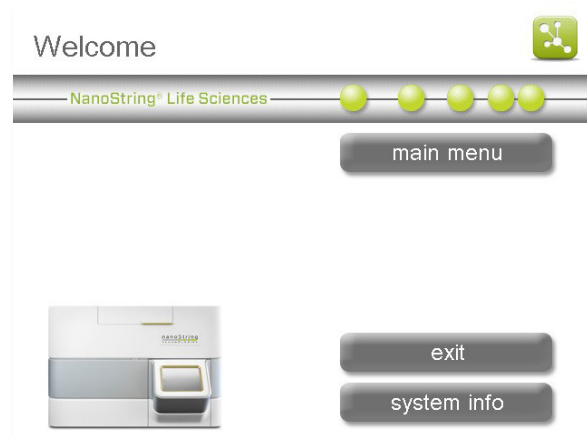
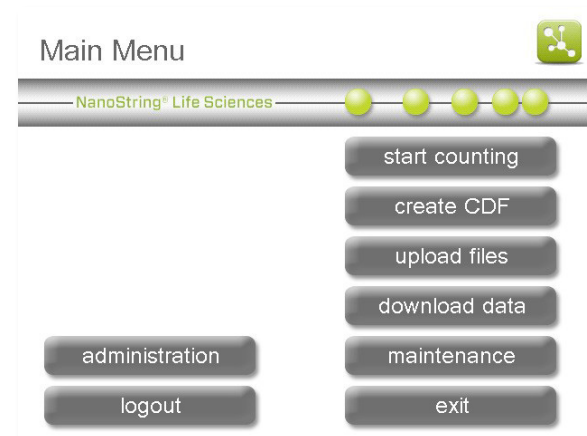**Figure 10:** The Digital Analyzer 'Welcome' screen.

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

Exit Life Sciences Mode

The user may exit Life Sciences mode by pressing 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.
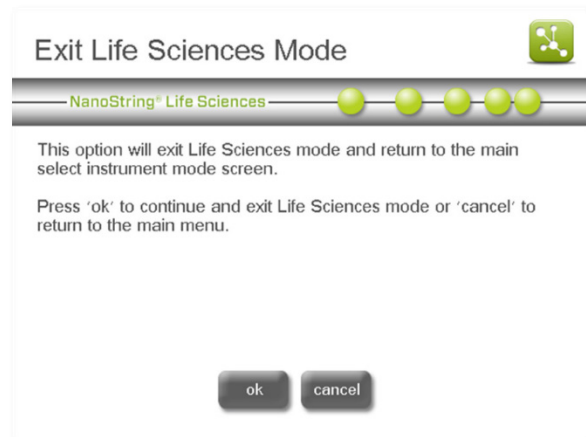

Figure 12: The Digital Analyzer 'Exit Life Sciences Mode' confirmation screen.

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.

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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-rxns, 48-rxns, or 192-rxns). 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 of Prep Station reagents; stored at 4°C; also referred to as Reagent Plates)

- One nCounter Cartridge (foil-sealed pouch; stored at -20°C)

- One nCounter Prep Pack (stored at room temperature)

    o   Racked Tips and Foil Piercers
    o   12-Tube Strips and Caps
    o   Tip Sheaths
    o   Cartridge Adhesive Covers
    o   Hybridization Buffer

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Initiating a Run

The following workflow begins after samples have been hybridized overnight, according to the protocol outlined in the user manual specific to the assay (such as the *nCounter miRNA Assay Manual* (MAN-C0009) or the *nCounter Gene Expression CodeSet RNA Hybridization Protocol* (MAN-10056)). 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, press **start processing** from the Main Menu (**Figure 13**).
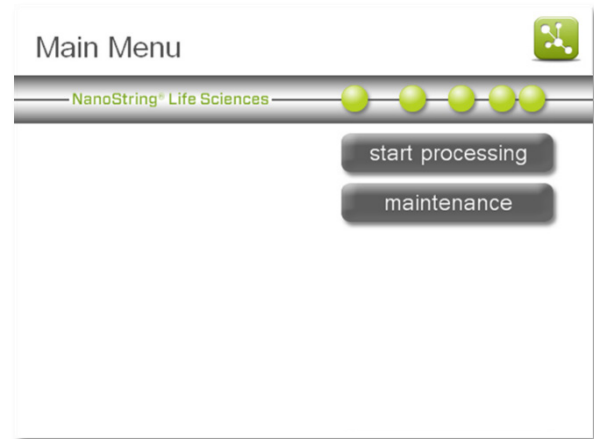
Figure 13: The Main Menu.

2. The 'Select Protocol' screen will appear (**Figure 14**). Select one of three protocols for processing samples:

    o   **High Sensitivity:** Recommended for most samples. Increases the binding of all molecules to the slide by ~2-fold and adds an extra 30 minutes to the processing time.
    o   **Standard:** Suitable for situations where the sample loaded per lane exceeds the recommended amount, where the experimental conditions are known to be near the saturation point of the instrument, or where the user is attempting to maximize the throughput of the instrument.

Figure 14: The 'Select Protocol' screen.

    o   **Legacy Protocol:** Consistent with Prep Station software versions released before 2015; can be used to maintain consistency on long-term projects. Used for a *Maintenance Dry Run*.

    NOTE: All protocols use the same reagents.

3. Press **next**.

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4. The 'Select sample tubes' screen will appear (**Figure 15**). Select the sample positions that will be processed. Green 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 enough reagents for 6 cartridge lanes. If processing more than 6 lanes, use two prep plates. If processing 6 lanes or less, use only one prep plate, placed in the front position, and ensure the hybridized samples are in tubes 1-6 of the 12-tube strip.
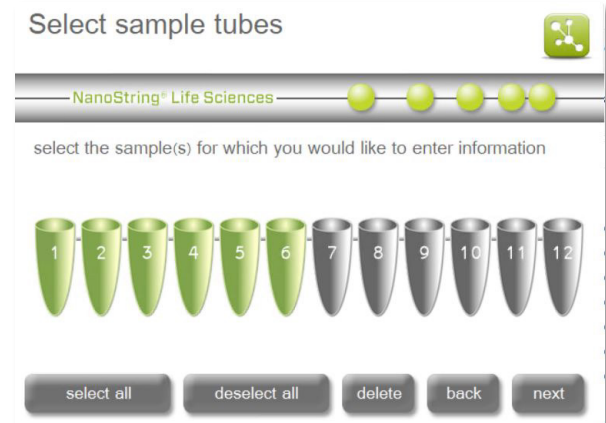
Figure 15: The 'Select sample tubes' screen.

   Any number of lanes between 1–12 will still consume an entire Cartridge.

5. The 'Warm Reagents & Cartridge' screen will appear (**Figure 16**). Cartridges and reagent plates (prep plates) (**Figure 17**) must be at room temperature prior to processing. Remove the 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.
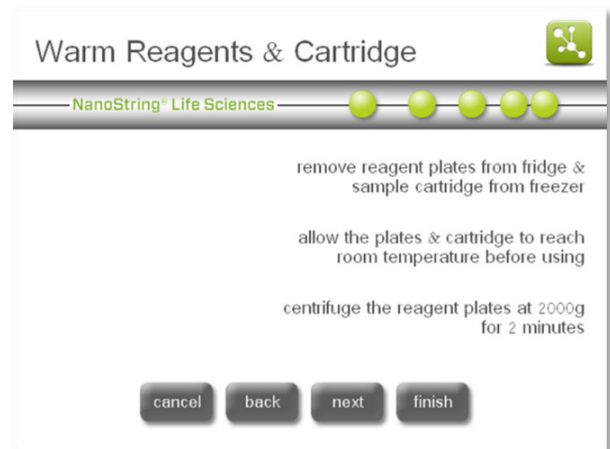
Figure 16: The 'Warm Reagents & Cartridge' screen.
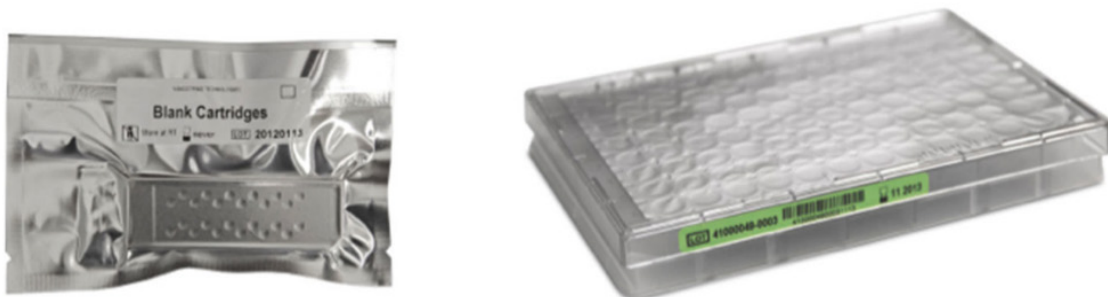
Figure 17: A sealed cartridge (left) and reagent plate ('prep plate') (right).

Centrifuge the prep plates at 2000g 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**.

6. 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**.
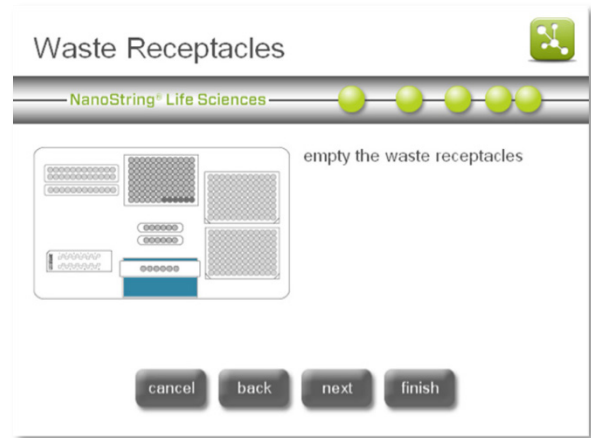
Figure 18: The 'Waste Receptacles' screen.

7. 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.
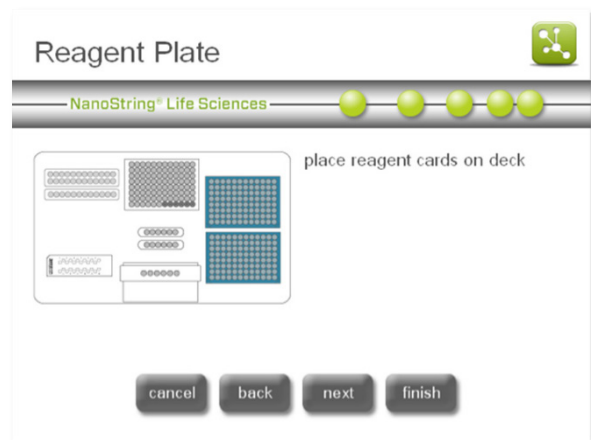
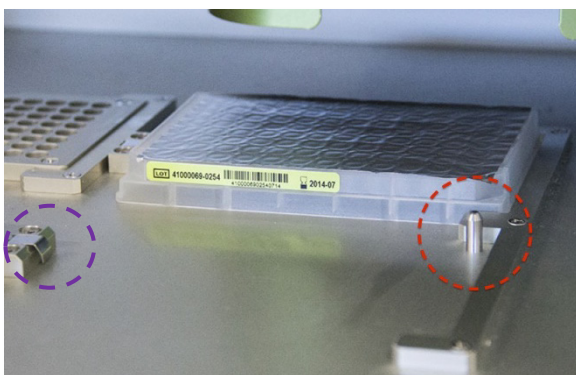**Figure 19:** The 'Reagent Plate' screen.
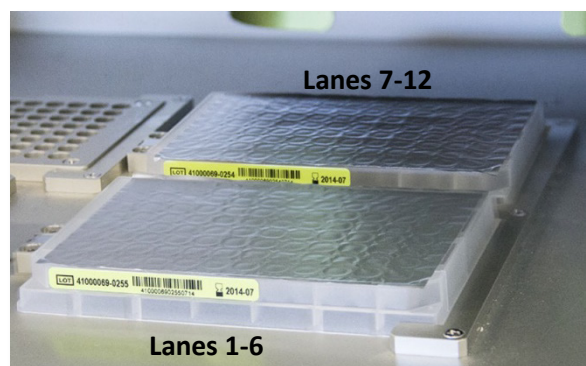
Figure 20: Use the positioning pin (circled in red for the front plate) to ensure correct placement of the Prep Plates. The plate catch (circled in purple) contains a spring clip that ensures the plate is positioned securely. Pressing down on the plate next to this spring clip will ensure the plate is flush with the deck.

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

8. 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. Each side of the box serves as 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.

    Press **next**.
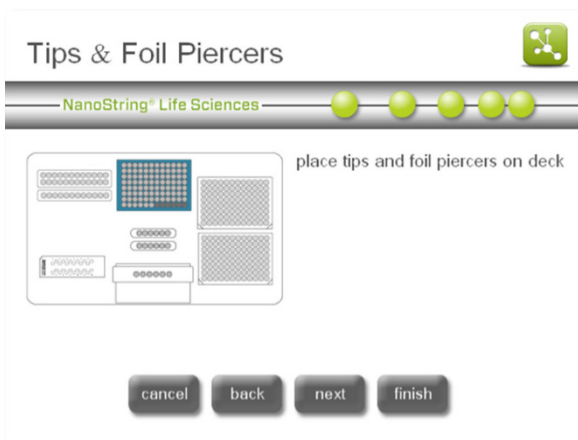
Figure 21: The 'Tips & Foil Piercers' screen.
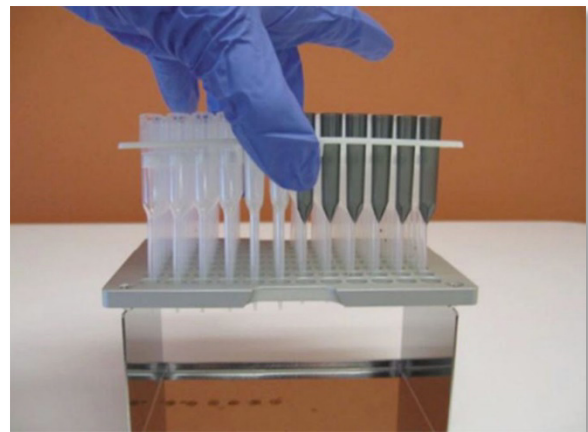
Figure 22: Placing the tips and foil piercers into metal tip carrier.

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

    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.

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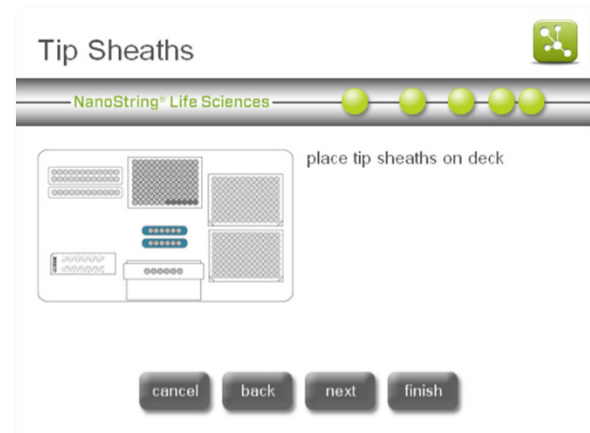
    Press **next**.

Figure 23: The 'Tip Sheaths' screen.

10. The 'Sample Cartridge' screen will appear (**Figure 24**). Carefully place a cartridge under the electrode fixture with the barcode to the right and logo to the left (**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**.
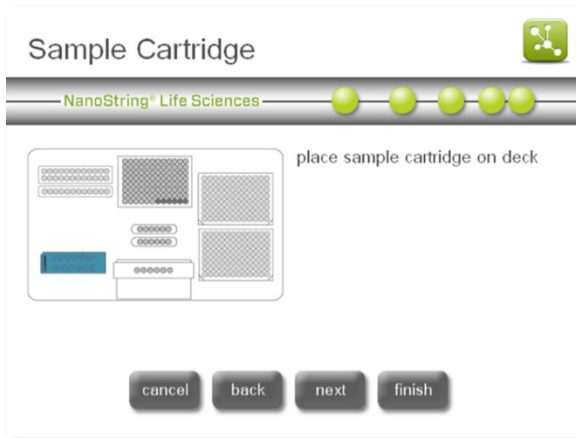

Figure 24: The 'Sample Cartridge' screen.

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

11. The 'Electrode Fixture' screen will appear (**Figure 26**). Slowly lower the electrode fixture into place over the cartridge using even pressure, until it clicks (**Figure 27**). The 24 electrodes should insert into the 24 wells. Stop if you encounter any resistance and check that all electrodes are clearing the wells before proceeding. 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**).
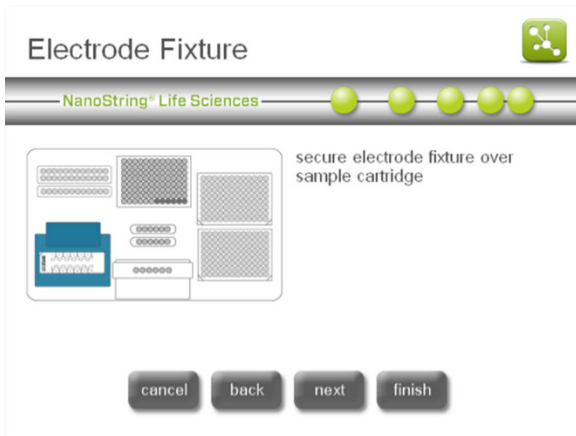

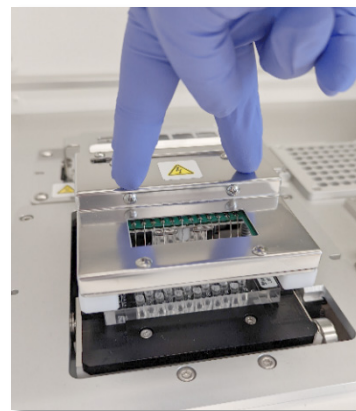Figure 26: The 'Electrode Fixture' screen.

Figure 27: Carefully lower the electrode fixture into place over the cartridge, until it clicks.

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.

12. 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.
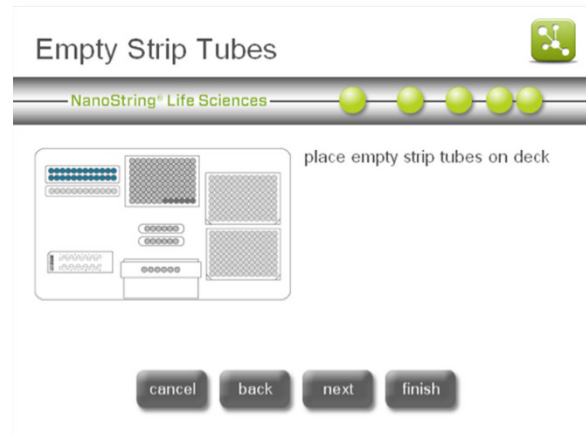
Figure 28: The 'Empty Strip Tubes' screen.

13. The 'Hybridized Samples' screen will appear (**Figure 29**). Lift the metal lid to place the hybridized sample strip tube in the sample strip holder, 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. Close the metal lid and press **next**.

    Hybridized sample strip tubes have two notches to ensure proper orientation (between tubes 1-2 and 8-9).

    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.
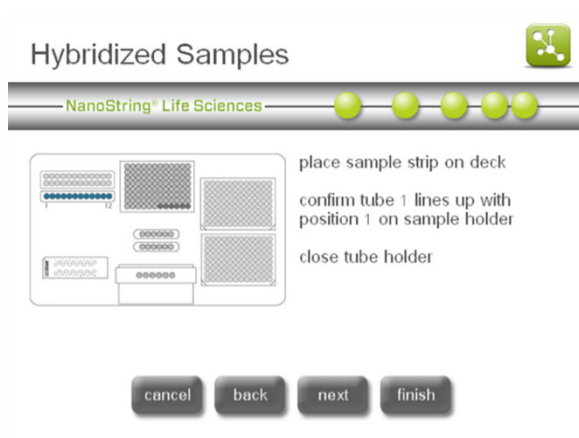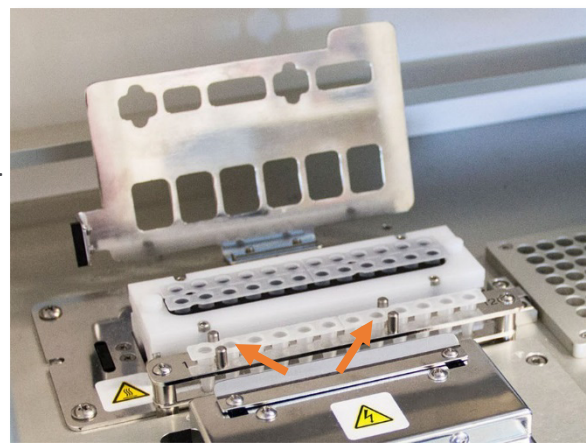
Figure 29: The 'Hybridized Samples' screen

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

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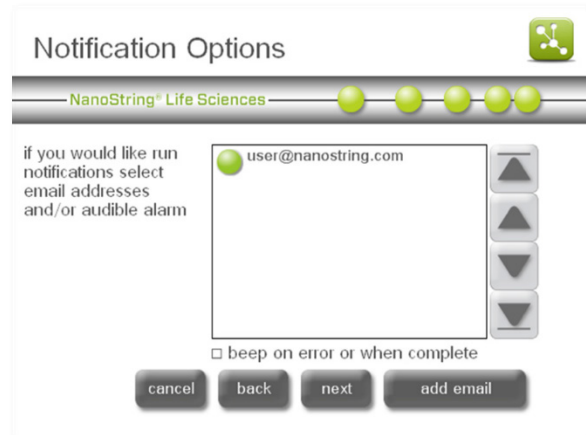

Figure 31: The 'Notification Options' screen.

15. Select the email addresses where error and completion confirmation emails 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.
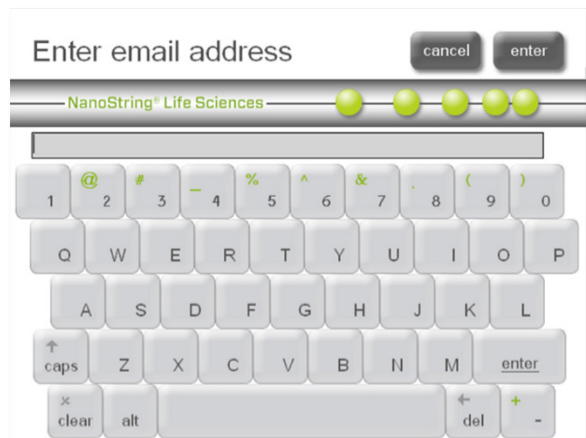

Figure 32: The 'Enter email address' screen.

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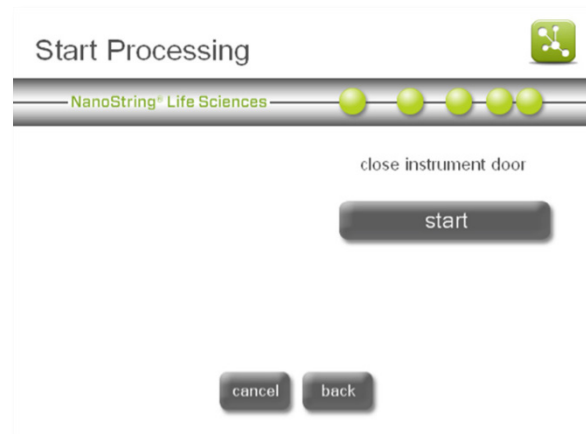

Figure 33: The 'Start Processing' screen.

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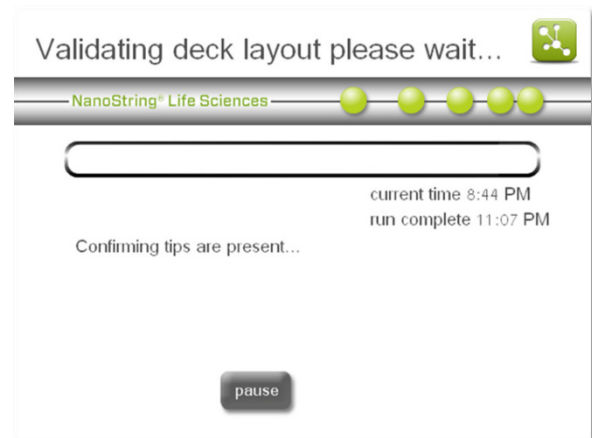

Figure 34: The 'Validating deck layout' screen.

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.

NOTE: Please stay with the Prep Station while the deck layout is being validated (~10 minutes). Any problems that may be encountered during this step will require user intervention to proceed.

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.

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Pausing or Aborting a Run

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.

1. 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**).

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.

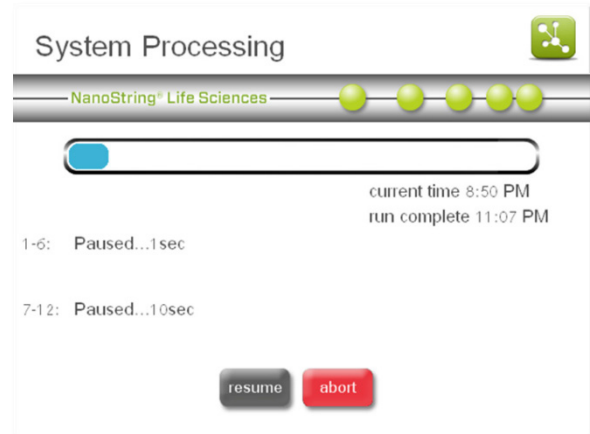2. Press **resume** to continue the run or press **abort** to end the run.

Figure 35: The paused 'System Processing' screen.

IMPORTANT: **When a run is aborted, the run cannot be restarted. Samples that have already been transferred or mixed with reagents cannot be recovered.**

When Run is Complete

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

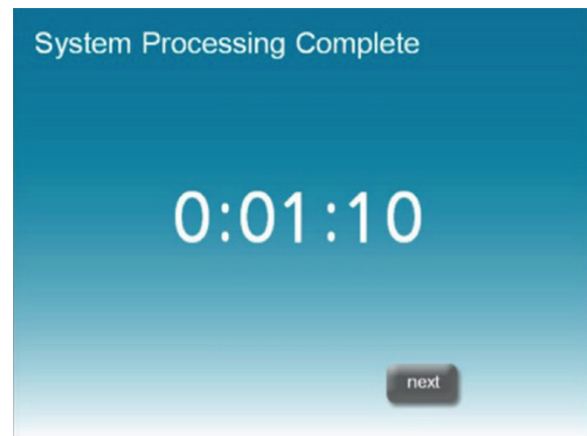1. Press **next**. The 'Run Successfully Completed' screen will appear (**Figure 37**).

Figure 36: The 'System Processing Complete' screen.

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2. 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 strip.

    d. Remove the sample cartridge and seal the wells.

Figure 37: The 'Run Successfully Completed' screen.

3. To release the fixture after the run is complete, lift the lever in the center top of the device towards the front with a finger as shown in **Figure 38**.

4. 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.

    c. Store samples 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.

Figure 38: Releasing the fixture after a completed run.

5. 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.
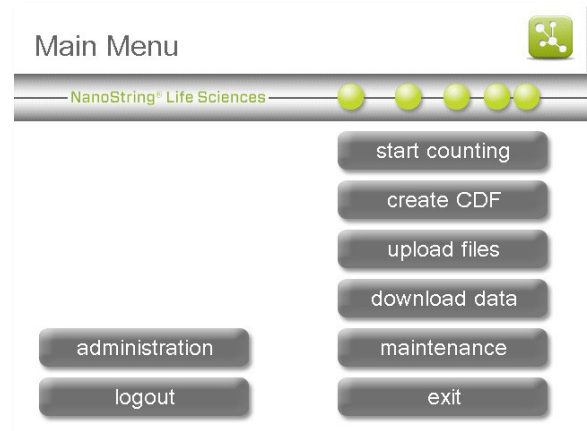

Figure 39: The Main Menu with the Enterprise Package enabled, and an administrator logged in.

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 user name "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 user name and password.

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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 log in before using the instrument.

- **USB/FTP settings:** Establish rules for uploading and downloading data by USB, FTPS, and email.

Figure 40: The Administration Menu.

- **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.

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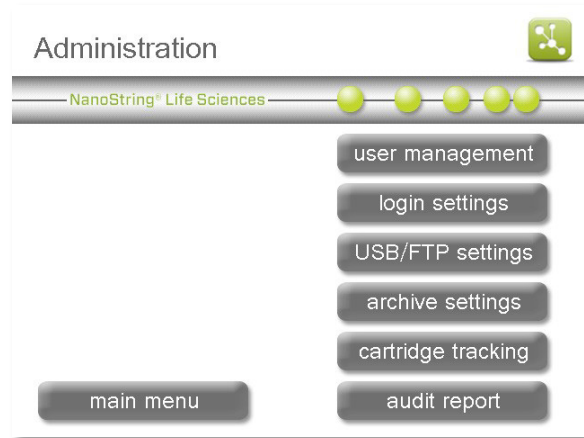
User Management

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.
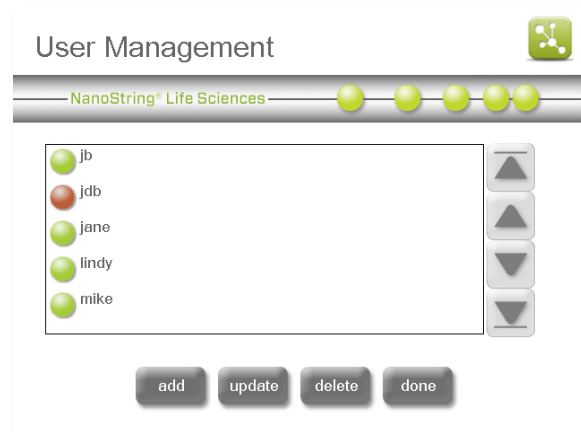

Figure 41: The 'User Management' screen.

Add a User

To create a new user, press the **add** button. The 'Create User' screen will appear (**Figure 42**).
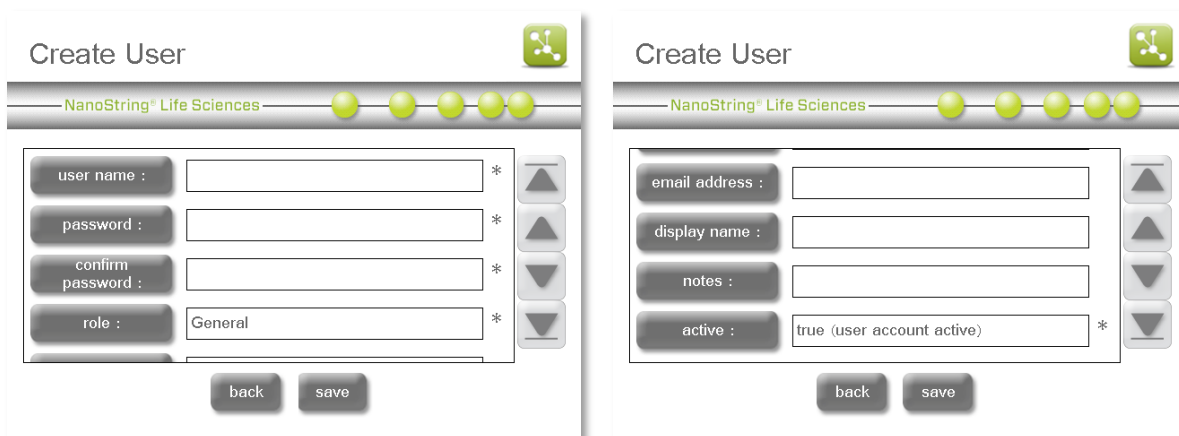
Figure 42: The 'Create User' screen: top half (left) and bottom half (right).

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 email address that will be associated with the User.

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- **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.

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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 ten-minute timeout will allow the instrument to wait ten 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.
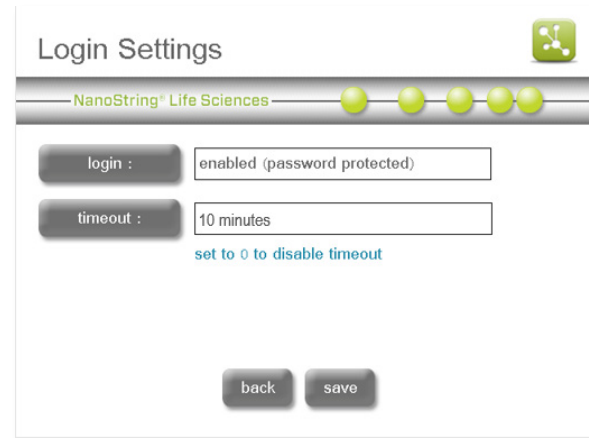
Figure 43: The Login Settings screen.

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, FTPS, 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.
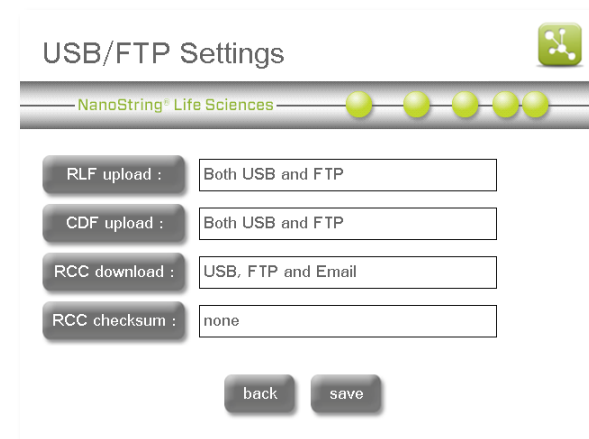
Figure 44: The USB/FTP Settings screen.

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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 FTPS. 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 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.
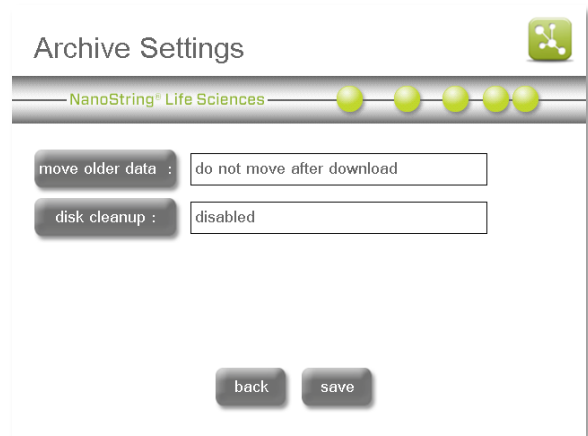
Figure 45: The Archive Settings screen.

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Cartridge Tracking

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'.

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

Figure 46: The Cartridge Tracking Settings screen.

Audit Report

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 FTPS 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.

Figure 47: The Audit Report Generation screen.

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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 in a cartridge, detailing the number of counts for each target in a lane.

Each CodeSet ships with a USB flash drive containing the following files for use on the Digital Analyzer. nCounter TagSets do not ship with a USB flash drive; the RLF is provided via email.

- Three folders for Cartridge Definition Files (CDFData), Reporter Library Files (RLFData), and Reporter Code Count files (RCCData) (**Figure 48**).

- A CDF template within the folder CDFData.

- A compressed file containing the nSolver AdvancedAnalysis Software installer and documentation. (nSolver Analysis software should be downloaded from the NanoString website).
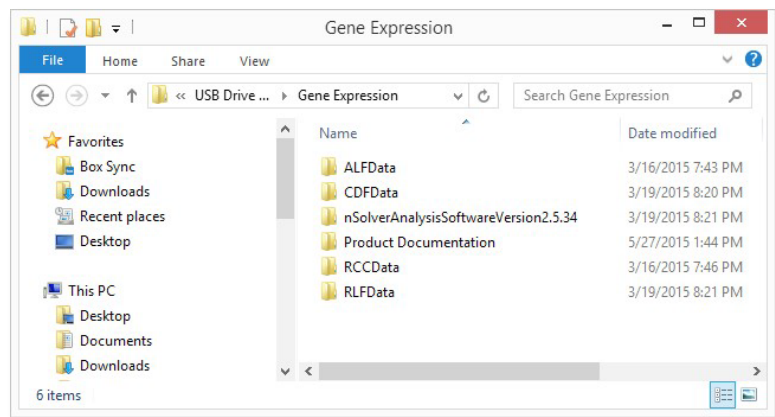
Figure 48: Example file folders stored on a USB flash drive.

- Product-specific documentation in PDF format.

- Material Safety Data Sheet(s).

Users may provide their own USB flash drive to transfer files but must create the same three folders (CDFData, RLFData, and RCCData) 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 or the system will not be able to locate the folder contents.

Navigating File/Folder Screens

The file/folder structure is similar to a personal computer with nested folder structures. Navigate down the folder hierarchy by double-tapping the folder to open it, similar to double-clicking a mouse (**Figure 49**). To navigate back to a higher-level folder, double tap the root directory in the top line 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.
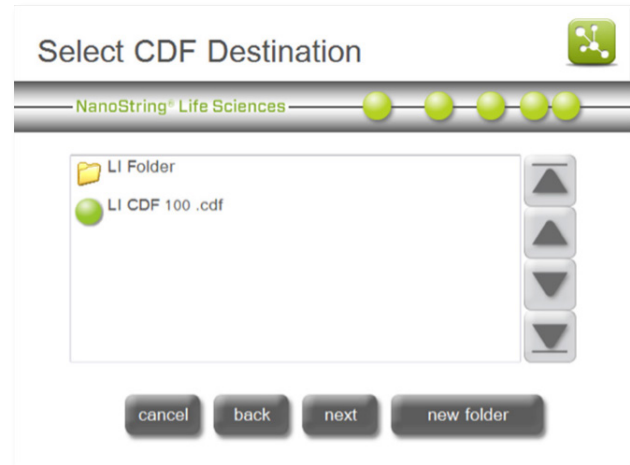
Figure 49: Navigating files and folders from the Digital Analyzer display.

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 FTPS (certain configurations may disable transfer by USB flash drive). The RLF corresponding to a specific CodeSet must be uploaded before setting up a run using that CodeSet. Once an RLF has been uploaded, it will always be available until it is manually deleted from the Digital Analyzer. 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.

2. From the Main Menu, press **upload files** (**Figure 50**).
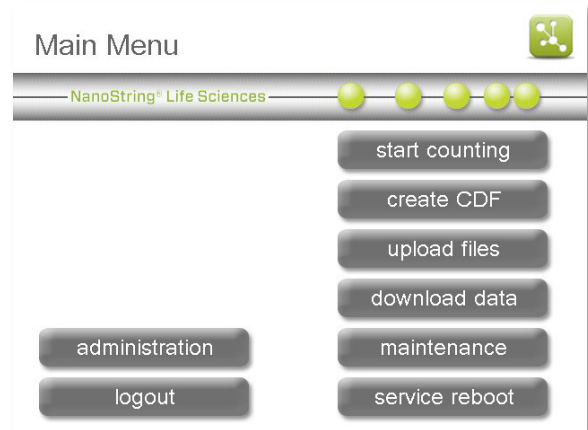
Figure 50: The Main Menu.

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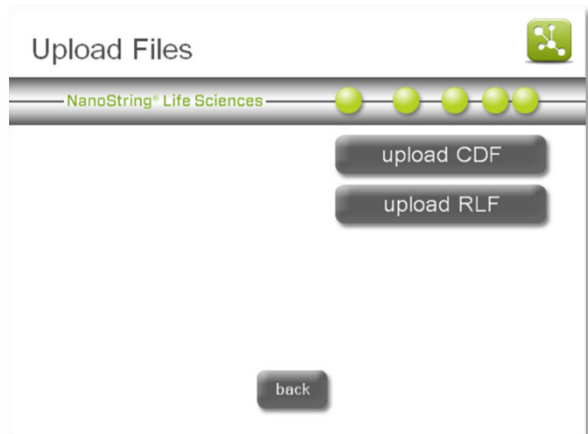
Figure 51: The 'Upload Files' screen

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.

4. Select one or more files and press **next** to upload the file(s).

The RLF will be saved on the Digital Analyzer.
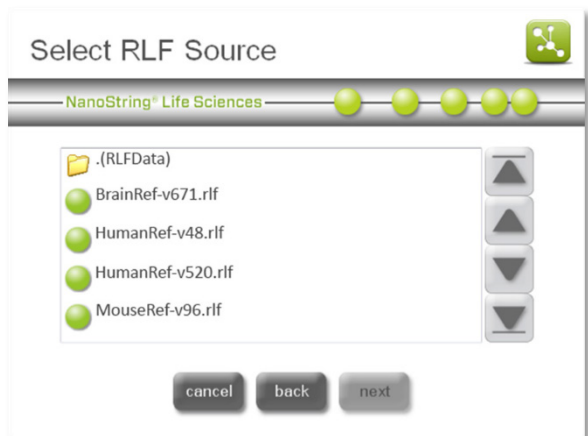
Figure 52: The 'Select RLF Source' screen

Upload RLFs via FTPS

Acquire the IP address, or instrument serial number, and FTPS 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 FTPS settings have been configured, RLFs may be transferred directly to the Digital Analyzer from a personal computer. There are many FTP Client applications available. For transferring data between the Digital Analyzer and a personal computer, the FTP client will need to support FTPS and TLS/SSL implicit encryption.

NOTE: Windows Explorer and Mac OS Finder do not support FTPS.

WinSCP is used for the following example steps and images.

1. On a Windows computer, launch the FTP-supported client and start a new connection with the Digital Analyzer IP address or serial number as shown in **Figure 53**. Ensure the following settings are equivalent in the FTPS client being used: Protocol = FTPS, Encryption = TLS/SSL implicit, Port = 990
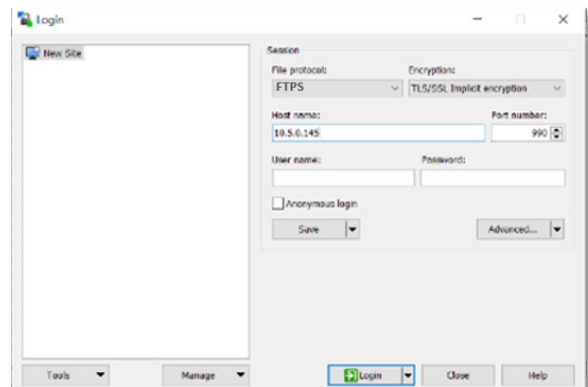
Figure 53: Enter the Digital Analyzer's IP address in the FTPS client connection.

2. Enter 'technician' as the FTPS user name as shown in **Figure 54**, 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**).
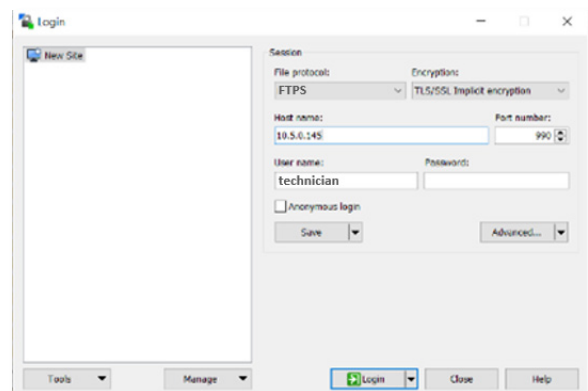
Figure 54: Enter 'technician' as the user name in the FTPS client connection.

3. Open the **RLFData** folder.

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

4. Copy and paste RLFs from the personal computer into the RLFData folder.
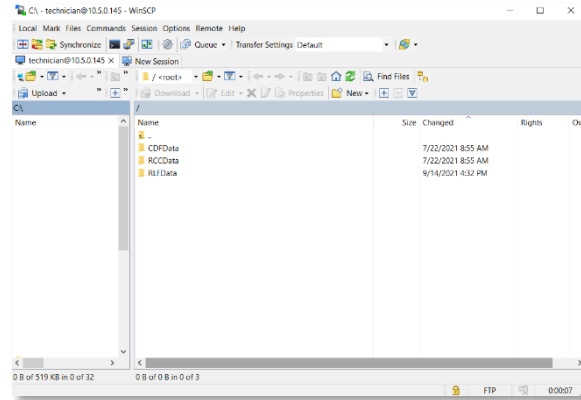
Figure 55: The contents of the instrument server when using an FTPS client.
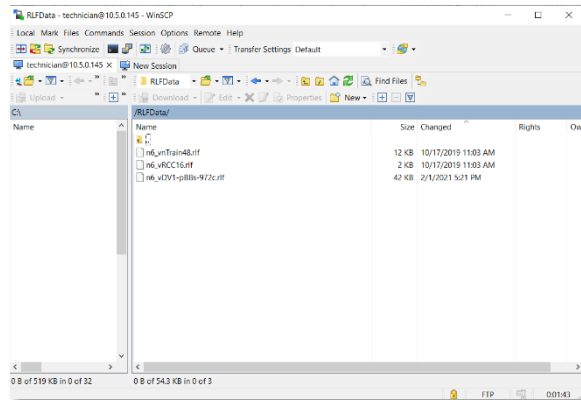

Figure 56: The contents of the 'RLFData' folder when using an FTPS client.

Cartridge Definition Files

A 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
LaneID	Defines which lanes in the cartridge will be scanned. If all twelve lanes will be scanned, then this should not be changed. If only a subset of the lanes will be scanned, then the information for the empty lanes can be deleted.
SampleID	Required. Sample identifiers can be specific names or assigned default names 01–12 corresponding to one or more of the 12 lanes 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	Optional. Example uses include the name of individual running the experiment or the study's Principal Investigator. Details are output within the RCC file as general information.
Comments	Optional. Example uses include details regarding the project or a specific sample. Details are output within the RCC file as general information.
Date	Optional. This field can contain any date in the YYYYMMDD format. The date of a scan is automatically added to the beginning of the RCC file name, so it is not required here.
FOVCount	Required. 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	Required. 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 CodeSet, so the correct RLF is critical. The ".rlf" file type extension should NOT be used here.

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 FTPS (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.

Figure 57: Select Sample Tubes screen

> IMPORTANT: When multiple tubes are selected, **all the info** showing on the screen **will overwrite 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.

A CDF can be created on a personal computer or the Digital Analyzer. Some examples are described below.

- **Scenario 1: Create a CDF using the CDF Utility on a personal computer.** This is the easiest and recommended method for creating CDFs. See *Creating a CDF Using the CDF Utility on a Personal Computer.*

- **Scenario 2: Create a CDF manually on a personal computer.** This is the most practical scenario if detailed and unique information is required for each sample within a cartridge, but macros cannot be enabled in Excel because of institution policies. See *Creating a CDF Manually on a Personal Computer.*

- **Scenario 3: Create a CDF on the Digital Analyzer and enter data for multiple tubes simultaneously.** This approach is very fast but will only allow identification of different samples by a unique lane number. Sample names will have to be lined up to lane numbers in the downstream analysis. 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.*

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Creating a CDF on a Personal Computer

> **IMPORTANT:** CDF(s) transferred to the Digital Analyzer on a USB flash drive **must** be inside a folder named **CDFData** to be recognized by the Digital Analyzer.

Creating a CDF Using the CDF Utility on a Personal Computer

Although a CDF can be generated manually on a computer, this manual process is error-prone due to formatting problems as well as keyboard characters that are not recognized by the Digital Analyzer (see section below). Because of these challenges, NanoString has created a macro-driven Excel file with built-in instructions as well as safeguards to prevent the more common sources of error. Download the CDF Utility from the NanoString webpage or NanoString University.

To create a CDF using the CDF Utility, open the file and click on **Enable Content** if the Security Warning pops up in Excel.
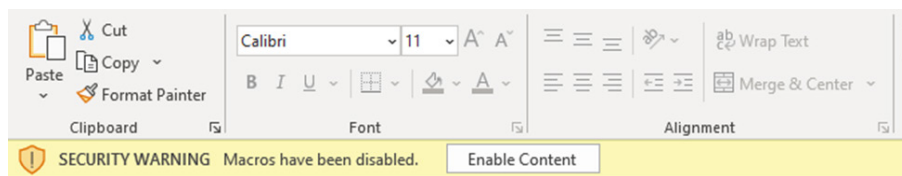
The Utility has protected those cells that should remain blank and prevents the use of keyboard characters that are not recognized by the Digital Analyzer. If you try to enter data in a protected cell or use an illegal character, a dialog box will yield a pop-up message indicating one of these two data entry errors.

The utility spreadsheet looks like:

Highlighted yellow cells indicate information is required, including CartridgeID, SampleID, FOVCount, and GeneRLF. Green cells are optional for additional information, including Email (if enabled on the Digital Analyzer), Owner, Comments, and Date (note that for tracking purposes the date of the scan will be automatically added to the start of each cartridge ID name as well as each sample File name). For information about each cell and the characters allowed, hover over a cell to summon a pop-up instruction:

To **create the CDF file**, complete the following:

1. Enter the **CartridgeID**.

2. (Optional) Enter the **Email** where the data should be sent.

3. Enter the **SampleID** for each of the 12 samples. These can be cut and pasted from another spreadsheet, but you will not be able to paste illegal characters into this field.

4. (Optional) Enter the **Owner**, **Comments**, and **Date** in their respective columns.

5. Click on the first cell in the **FovCount** column. Using the drop-down menu, select the desired Scan resolution for the first cell (usually 555 or 280 FOV). Click the Copy-Down button just above the FovCount column to fill this choice for all 12 rows.

6. Click on the first cell in the **GeneRLF** column; enter the name of the RLF corresponding to the probes on this cartridge. In most cases, users will be running 12 samples from the same CodeSet on each cartridge, and therefore the same RLF should be copied and pasted into the 12 yellow cells of this column.

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IMPORTANT: The name entered in the GeneRLF column must be identical to the RLF loaded into the scanner. To prevent errors in transcribing, simply copy and paste the name from the RLF itself. Navigate to the RLF location using File Explorer (PC) or Finder (Mac):

NS_CancerImmune_V1.1.rlf
NS_CancerPath_C2535.rlf
NS_CancerProg_C3418.rlf
NS_CancerProg_C4972.rlf
NS_Hs_AD30_v1.0.rlf

a. Left click the RLF name once:

NS_CancerImmune_V1.1.rlf
NS_CancerPath_C2535.rlf
NS_CancerProg_C3418.rlf
NS_CancerProg_C4972.rlf
NS_Hs_AD30_v1.0.rlf
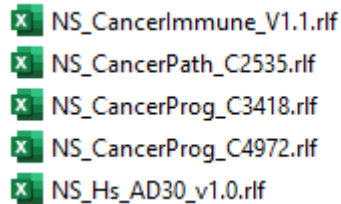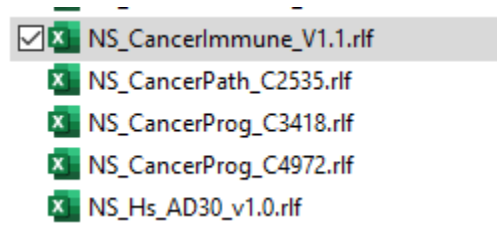
b. Then after waiting a few seconds, left click the RLF name again:

NS_CancerImmune_V1.1.rlf
NS_CancerPath_C2535.rlf
NS_CancerProg_C3418.rlf
NS_CancerProg_C4972.rlf
NS_Hs_AD30_v1.0.rlf
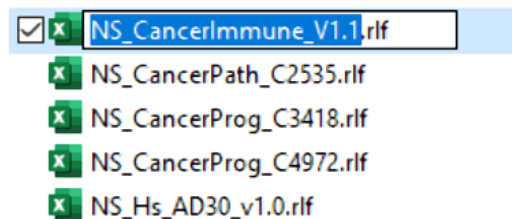
c. Now Copy (*Ctrl c*) this text and paste it into the cells G10 through G21 of the CDF utility spreadsheet. Note that the RLF name will usually contain an underscore character (_), but this character is allowed in this field in the CDF (and are required to recognize the correct RLF name). Do not include the .rlf extension when transferring this information to the CDF utility spreadsheet.

7. After all the required (yellow) cells have been completed, left click on the button labeled **Create Cartridge Definition File (CDF)** underneath row 21. This will generate a comma-delimited text file with the appropriate *.cdf* extension.

8. Save or transfer the file to a USB flash drive; it **must** reside in the **CDFData** folder on the USB in order to be located by the Digital Analyzer.

NOTE: For facilities that do not allow transfer with USB drives, transfer can be performed via FTPS, see *Upload CDFs via FTPS* for more details.

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Creating a CDF Manually 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 received your RLF in an email from Bioinformatics, that email will also contain the CDF template that comes on the USB drive for CodeSets. Although this works for generating CDF files, it can be prone to errors due to formatting or using restricted characters. Therefore, it is recommended to use the CDF Utility tool to generate CDF files on a personal computer (see *Creating a CDF Using the CDF Utility on a Personal Computer*).

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.
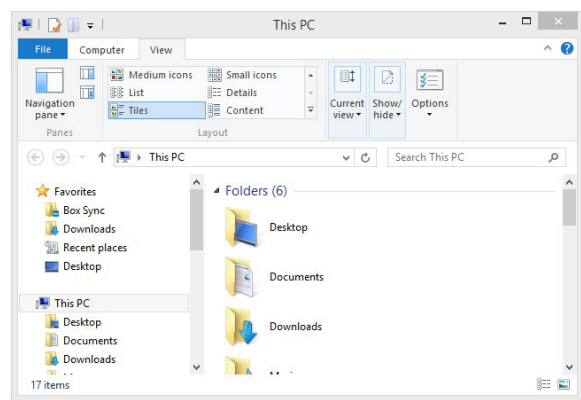
Figure 58: Location of the Options button.

3. 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**.
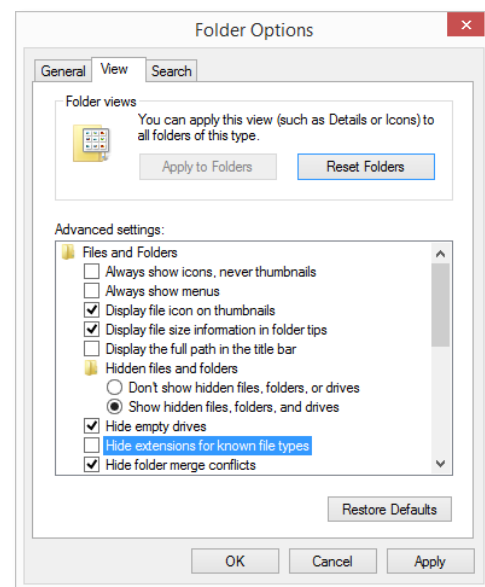
Figure 59: The 'Folder Options' window.

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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 lane per row
of the CDF.

5. When complete, save to the **CDFData** folder as file type ".csv"; the file name will include BOTH ".cdf" and ".csv" extensions as shown in **Figure 61, top**.

6. Close the file.

7. Remove the extra ".csv" file extension, leaving the file extension ".cdf" (**Figure 61, bottom**). If a warning appears, click **Yes** to continue.

IMPORTANT: The CDF must be placed in the **CDFData** folder on the USB flash drive for the Digital Analyzer to find it.
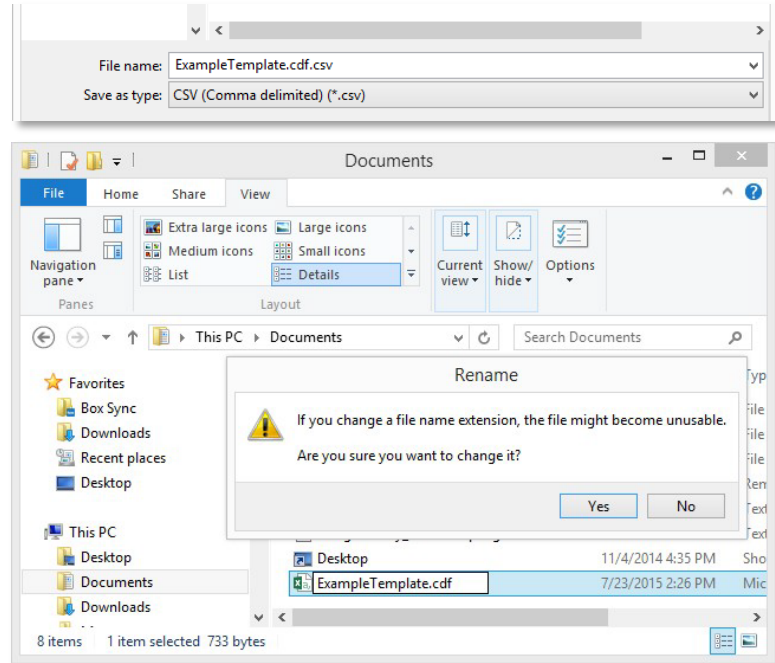
Figure 61: Removing the ".csv" file extension.

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Modify an Existing CDF in Notepad

NOTE: To avoid errors in file content and format. this method is not recommended unless necessary.
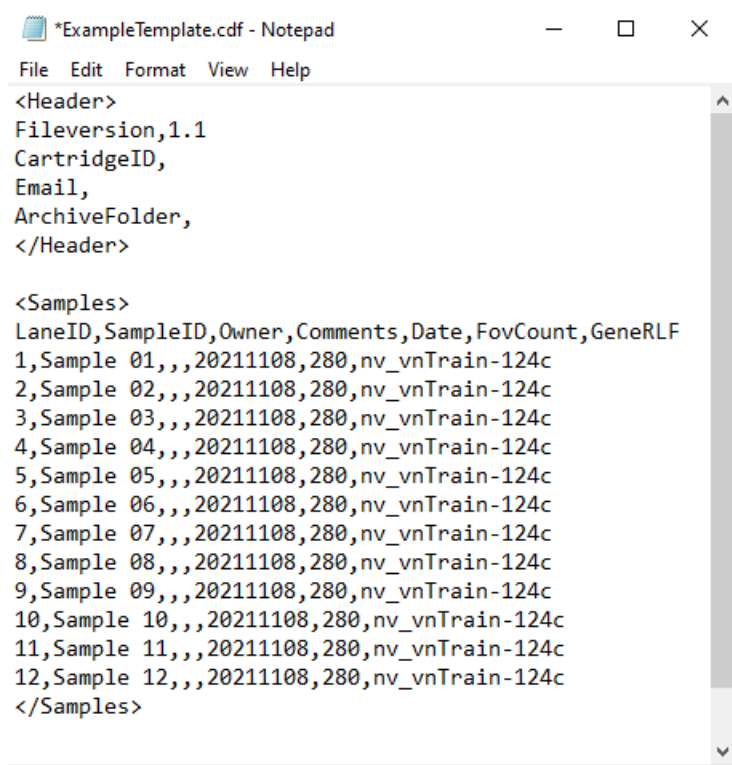
1. Open the CDF in Notepad (or TextEdit). Note that all the information present in the Excel spreadsheet is also visible in Notepad (**Figure 62**).

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 Email 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.

Figure 62: A sample CDF viewed in Notepad.

IMPORTANT: All 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 Flash Drive

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 FTPS, 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**).
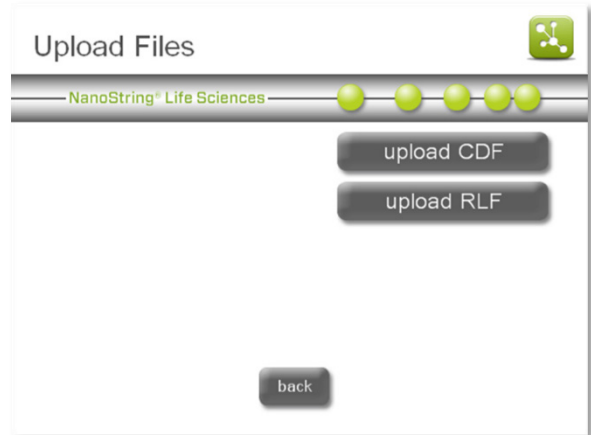
Figure 63: The 'Upload Files' screen.

3. Select **upload CDF**. The 'Select CDF Source' screen will appear (**Figure 64**).

4. Browse to the **CDFData** folder on the USB flash drive and select the desired CDF. The CDF must be in the **CDFData** folder to be recognized by the Digital Analyzer. Press **next**.
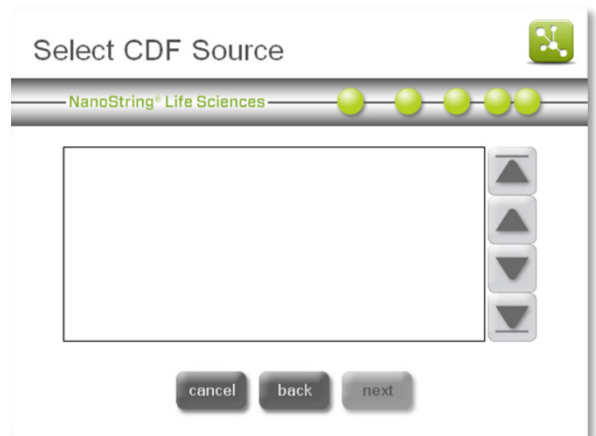

Figure 64: The 'Select CDF Source' screen.

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5. The 'Select CDF Destination' screen will appear (**Figure 65**). Browse to the folder CDFData and press **save**.
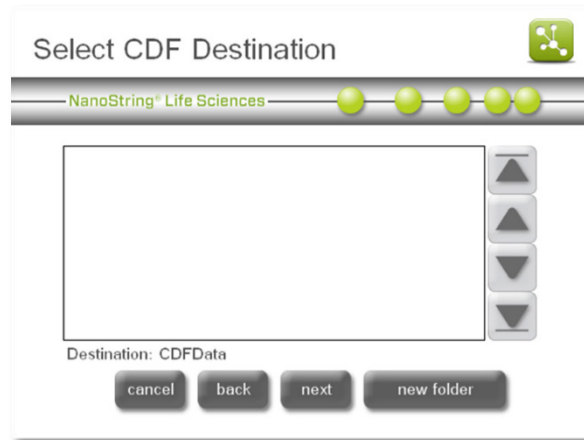

Figure 65: The 'Select CDF Destination' screen.

Upload CDFs via FTPS

Acquire the IP address, or instrument serial number, and FTPS 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 FTPS settings have been configured, CDFs may be transferred directly to the Digital Analyzer from a personal computer. There are many FTP applications available. For transferring data between the Digital Analyzer and a personal computer, the FTP client will need to support FTPS and TLS/SSL implicit encryption.
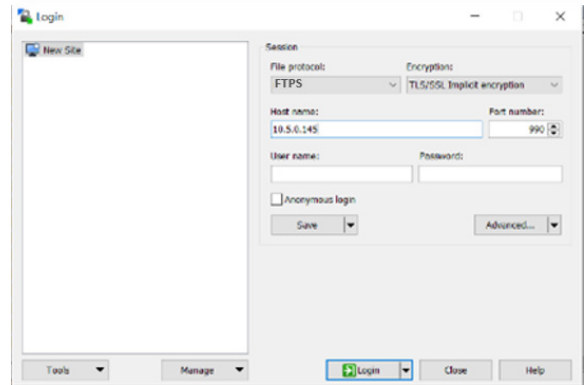

Figure 66: Enter the Digital Analyzer's IP address in the FTPS client connection.

NOTE: Windows Explorer and Mac OS Finder do not support FTPS.

WinSCP is used for the following example steps and images.

1. On a Windows computer, launch the FTPS-supported client and start a new connection with the Digital Analyzer IP address or serial number as shown in **Figure 66**. Ensure the following settings are equivalent in the FTP client being used: Protocol = FTPS, Encryption = TLS/SSL implicit, Port = 990

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2. Enter 'technician' as the FTPS user name as shown in **Figure 67**, enter the password as defined in the maintenance menu, and then click **Log On** or **Connect**.
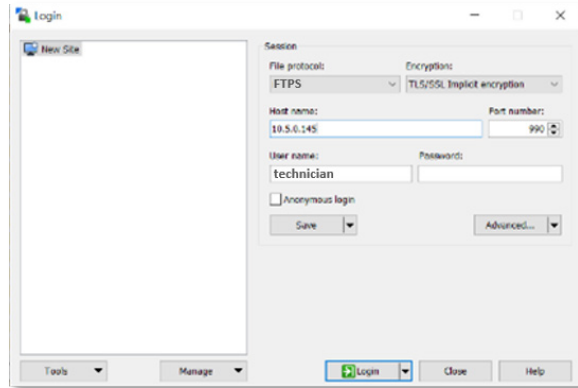

Figure 67: Enter 'technician' as the user name in the FTPS client connection.

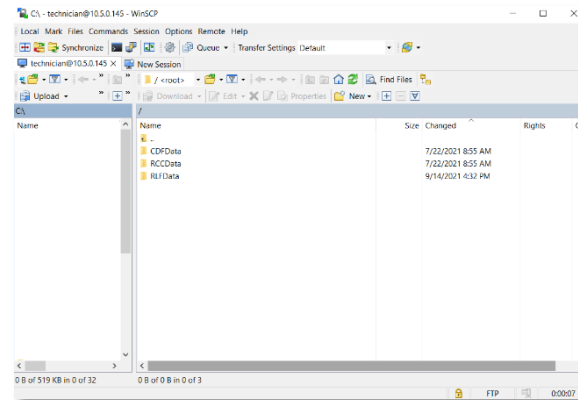The contents of the instrument server will appear (**Figure 68**).

Figure 68: The contents of the instrument server when using an FTPS client.

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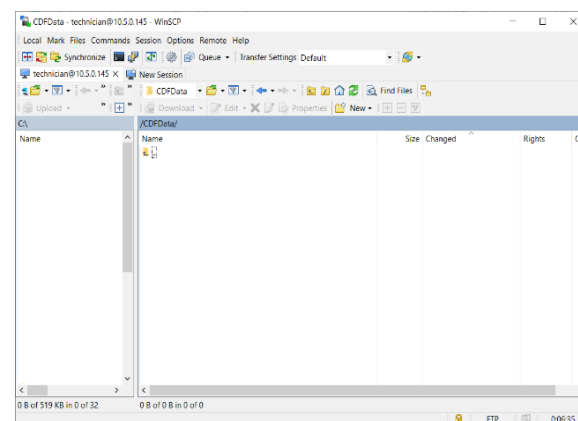

4. Copy and paste CDFs from the personal computer into the CDFData folder.

Figure 69: The contents of the 'CDFData' folder when using an FTP client.

Creating a CDF on the Digital Analyzer

The fastest method for 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 creating a CDF on the Digital Analyzer, including how to input unique identifying information for each sample.

1. From the Main Menu, 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**).

3. Enter the cartridge ID using the touchscreen 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.

Figure 71: The 'Enter cartridge ID' screen.

4. The Digital Analyzer will return to the 'Cartridge information' screen (**Figure 72**). Press **fov count** to change the data resolution.

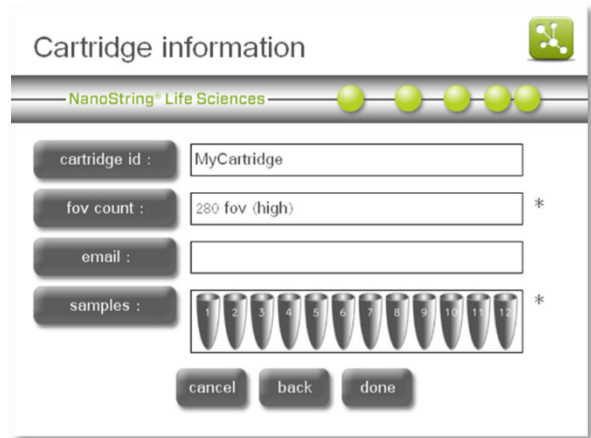The 'Select Data Resolution' screen will appear (**Figure 73**).

Figure 72: The 'Cartridge information' screen

5. 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 (lane) *vs.* 20 minutes per flow cell at maximum resolution.
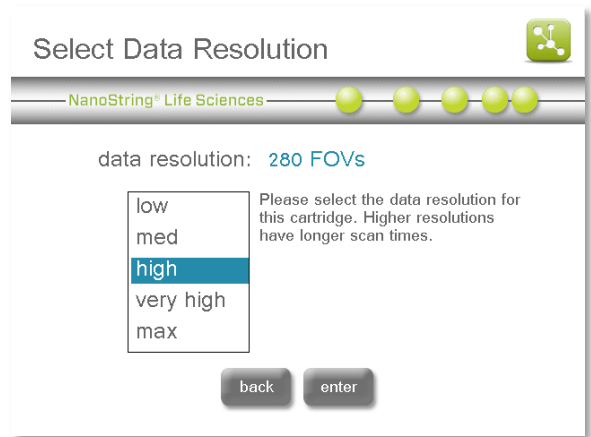

Figure 73: The 'Select Data Resolution' screen

Resolution	Images per sample
low	25
medium	100
high	280
very high	490
max	555

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.

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

7.   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 the 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.
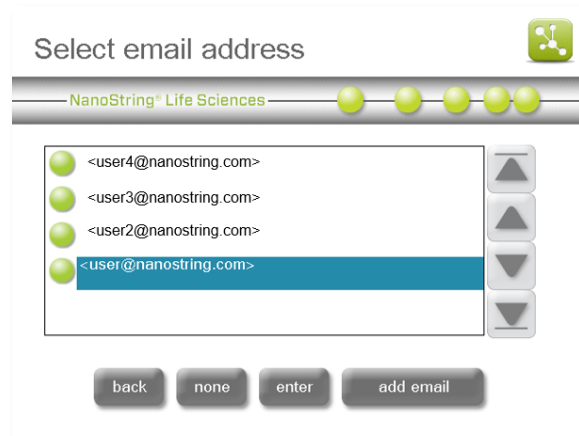
Figure 74: The 'Select email address' screen.

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

NOTE: Email must be configured first 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*.

8.   Press **enter**. The system will return to the 'Cartridge information' screen (**Figure 75**).

9.   Select the **samples** field. The 'Select sample tubes' screen will appear (**Figure 76**).
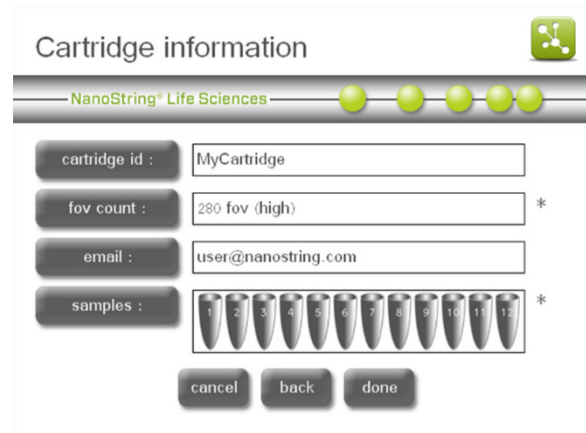
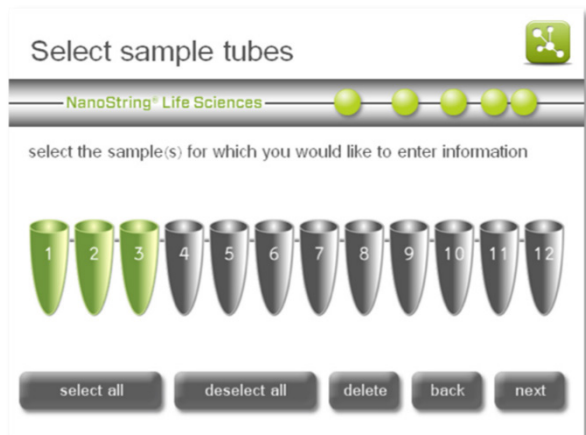**Figure 75:** The 'Cartridge information' screen.

Figure 76: The 'Select sample tubes' screen.

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10. Select the tubes for which sample information will be entered by pressing 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).

Figure 77: The 'Sample tubes' screen.

11. On the 'Sample tubes' screen, the available sample definition fields are displayed. Select each button or field 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 desired 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.

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

12. Once information for selected fields is input, 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**).

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**.

Figure 79: The 'Cartridge information' screen.

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

13. Select an existing folder or create a new folder in which to save the CDF. Press **next**.

Figure 80: The 'Select CDF Destination' screen.

The 'Enter new cdf name' screen will appear (**Figure 81**).

14. Enter the CDF name using the screen's keyboard and press **enter**.

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**).

15. 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 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 tapping that cartridge position on the screen.

   The selected cartridge will appear in green. If the wrong cartridge is selected, tap the correct position to change your selection. Press **next**.

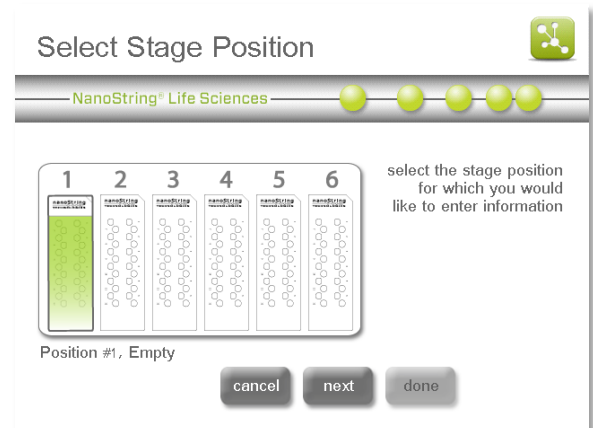**Figure 83:** The Main Menu.

Figure 84: The 'Select Stage Position' screen.

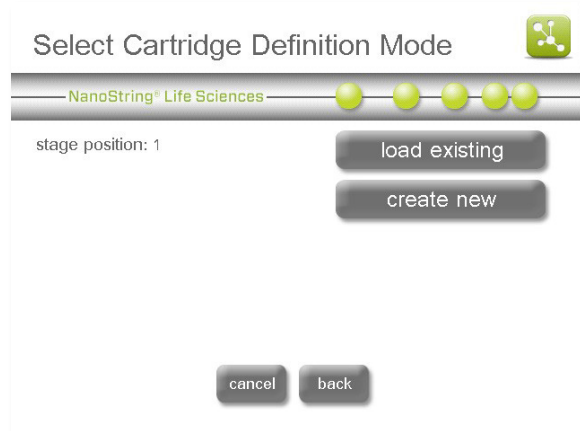3. The 'Select Cartridge Definition Mode' screen will appear (**Figure 85**).

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

4. 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).

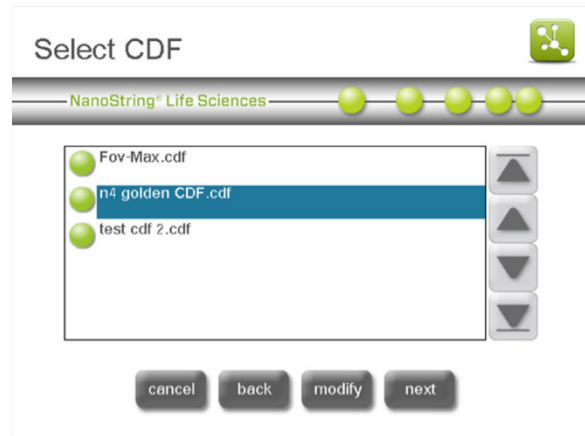   After pressing **load existing**, the 'Select CDF' screen will appear (**Figure 86**).

Figure 86: The 'Select CDF' screen.

5. 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.**
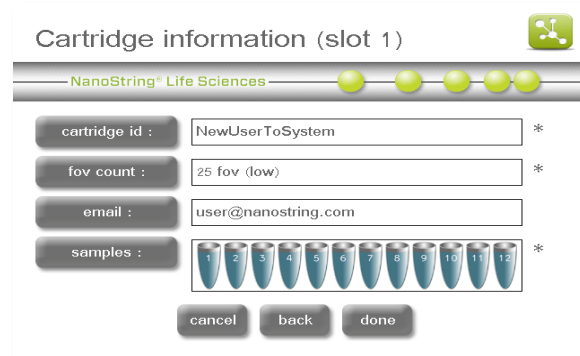

Figure 87: The 'Cartridge Information' screen, displaying details for slot 1.

6. To change any of the information specified for the cartridge, tap 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**).

8. For additional cartridges, press **next** to repeat **Steps 2–7** above until all cartridges for the run are defined.
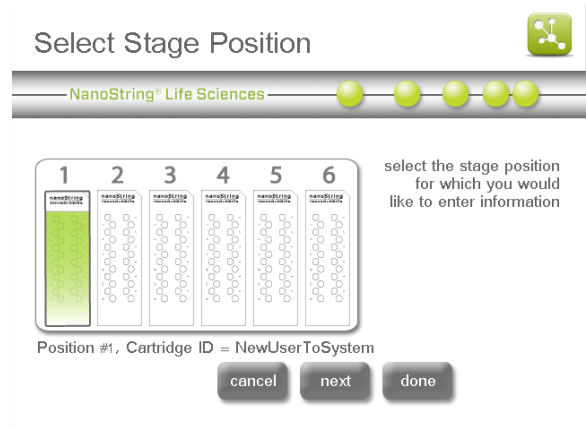
9. Press **done**.

Figure 88: The 'Select Stage Position' screen.

10. Place cartridges into the stage position corresponding to the data entered in **Steps 2–7** above. Use the orientation shown in **Figure 89**, with the barcode closest to the front of the instrument. Be sure that each cartridge is seated flat in the slot. Close the magnetic clips gently.
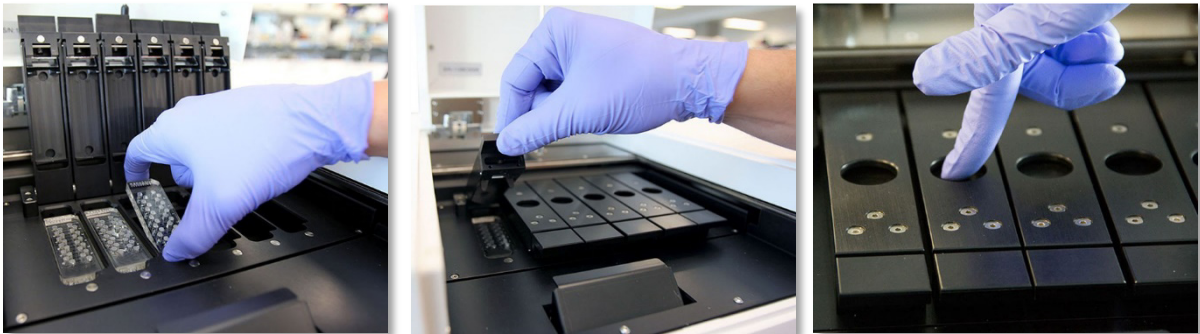

Figure 89: Proper orientation of cartridges when placing them into the Digital Analyzer. Close the magnetic clips gently and be sure to push down on the metal plate above the cartridge after the lid is closed to ensure the cartridge lays flat.

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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

  o **Blue:** cartridge scan completed and/or in progress
  o **Green:** cartridge yet to be scanned during the run
  o **Clear (white):** cartridge position for which no data has been defined

- Real-time data on the status of data collection

  o **current time:** the current time of day as defined in the system setup utility
  o **time left (#):** the approximate amount of time to complete the active cartridge
  o **time left (all):** the amount of time to complete all cartridges
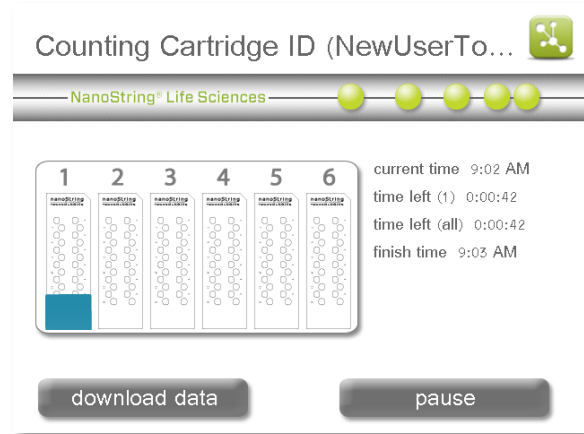 o **finish time:** the time of day the run will be finished

12. 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**).

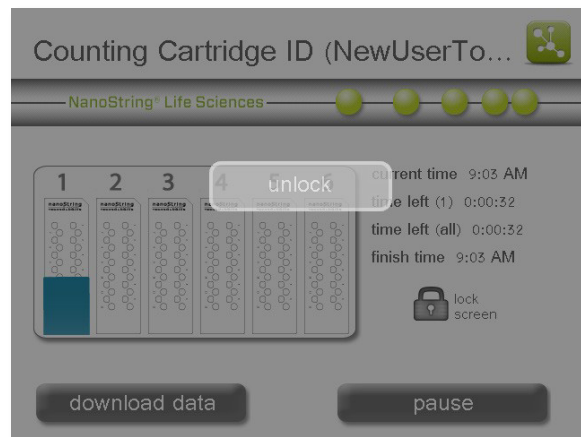**Figure 90:** The 'Counting Cartridge ID' screen with information on the current run.

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

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.

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

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

Figure 93: The 'Counting Cartridge ID' screen with completed run information.

Pausing a Run

From the 'Counting Cartridge' screen (**Figure 92**), 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.
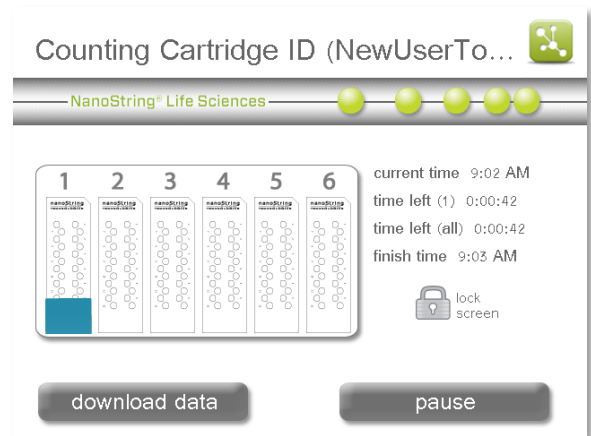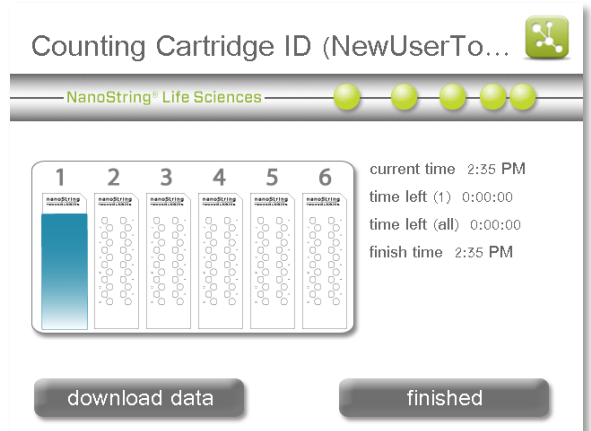
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NOTE: The **pause** button is present while the run is still in progress. Once the run is complete, the **pause** button will be replaced by the **finished** button.

Add a New Cartridge

1. On the 'Counting Cartridge ID' screen, press **pause**.

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

2. Press **add cartridge**.

3. 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.
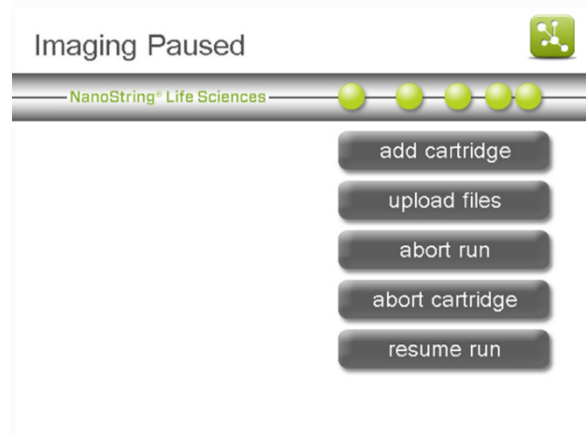
Figure 94: The 'Imaging Paused' screen.

Abort a Run

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

1. On the 'Counting Cartridge ID' screen, 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**).
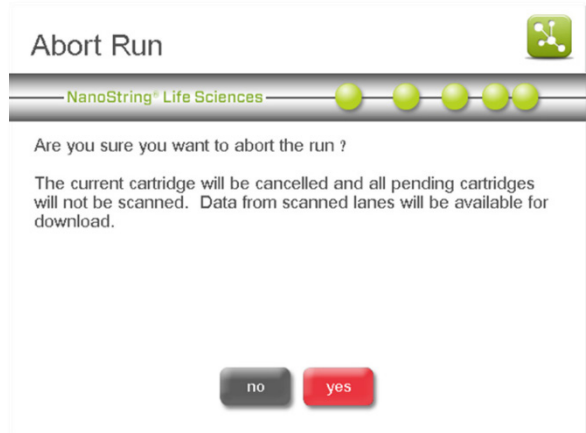
3. Press **yes** to abort the run.

Figure 95: The 'Abort Run' confirmation screen.

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 lanes that have completed imaging are saved and will be available for download.

Figure 96: The 'Abort Cartridge' confirmation screen.

1. In the 'Counting Cartridge ID' screen, 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**).

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.

Figure 97: The 'Upload Files' screen.

1. On the 'Counting Cartridge ID' screen, press **pause**.

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

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

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

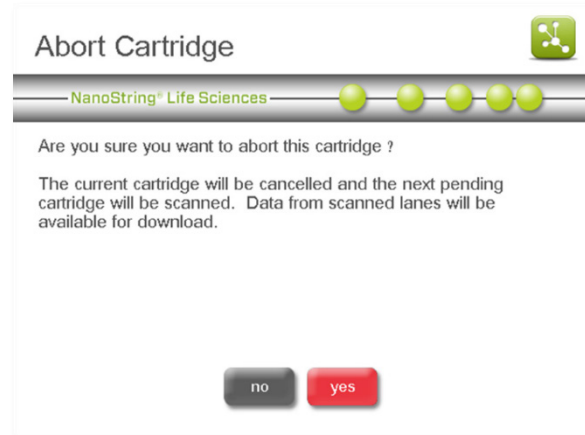4. Follow the instructions to upload files located in the *Cartridge Definition Files* section.

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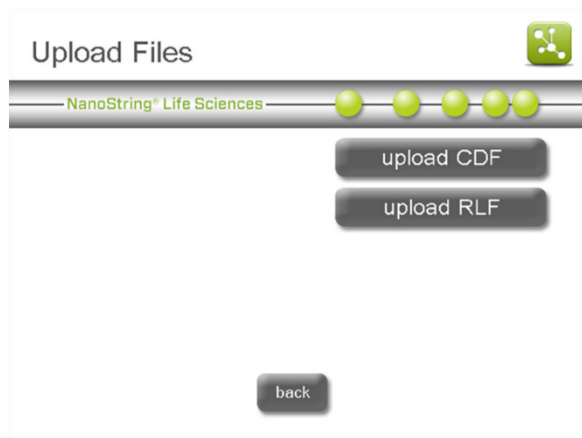
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Completing a Run

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

A Reporter Code Count (RCC) file is created for each lane 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 (SFTP) transfer to a personal computer

- FTPS transfer to a personal computer

Download RCC Files via USB Flash Drive

Figure 98: The Main Menu.

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 Folder.' See the *Enterprise Package* section for more information.
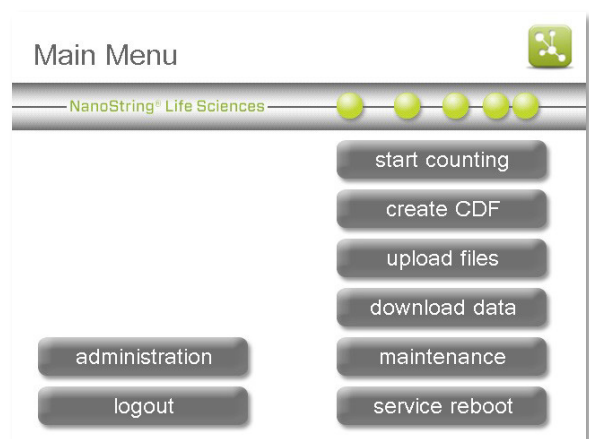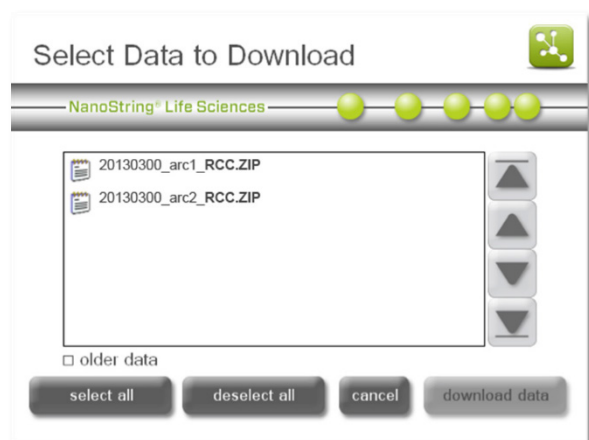

Figure 99: The 'Select Data to Download' screen.

3.   To select a file, tap the file name on the screen. One or more files may be selected. Selections will turn blue.

4.   To de-select a file, tap it again. The deselected files turn from blue to white.

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

Selecting "Older Data" Files

If data files cannot be found 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.

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 tapping it twice and select only the cartridge of interest. To go back to the top-level directory, press **..(Recycle Bin)** twice. Files are not removed from the Older Data folder unless manually removed.

Download RCC Files via Email

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 in email.
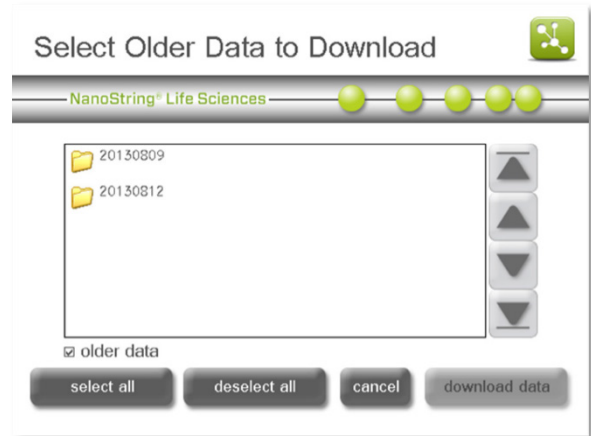
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Download RCC Files via SSH (SFTP)

If SSH (SFTP) rather than FTPS is preferred to transfer data files, this can be configured on the Digital Analyzer by NanoString personnel. Contact NanoString Support at support@nanostring.com to have SSH (SFTP) configured and enabled on the Digital Analyzer.

Download RCC Files via FTPS

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

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

If the instrument FTPS settings have been configured, RCC files may be transferred directly to a personal computer from the Digital Analyzer. There are many FTP applications available. For

Figure 101: The 'System Information' screen.

transferring data between the Digital Analyzer and a personal computer, the FTP client will need to support FTPS and TLS/SSL implicit encryption.

> NOTE: Windows Explorer and Mac OS Finder do not support FTPS.

WinSCP is used for the following example steps and images.

1. On a Windows computer, launch the FTPS-supported client and start a new connection with the Digital Analyzer IP address or serial number as shown in **Figure 102**. Ensure the following settings are equivalent in the FTP client being used: Protocol = FTPS, Encryption = TLS/SSL implicit, Port = 990.
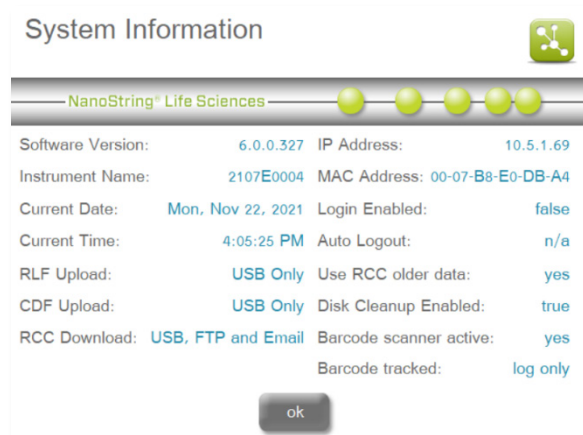

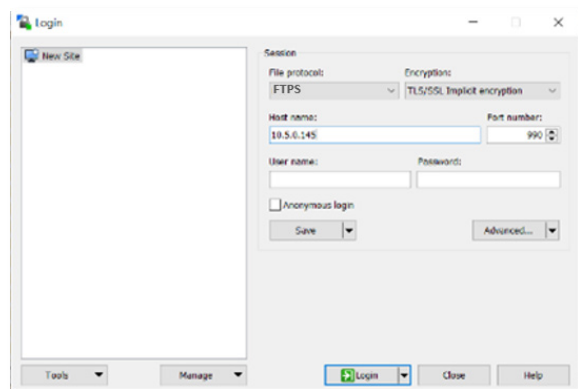**Figure 102:** Enter the Digital Analyzer's IP address in the FTPS client connection.

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2. Enter 'technician' as the FTPS user name as shown in **Figure 103**, enter the password as defined in the maintenance menu, and then click **Log On** or **Connect**.

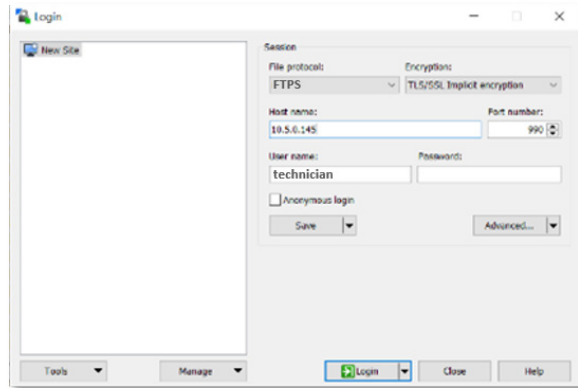   The contents of the instrument server will appear (**Figure 104**).

Figure 103: Enter 'technician' as the user name in the FTPS client connection.

3. Open the **RCCData** folder. The RCCData folder will open and display the available files (**Figure 105**).
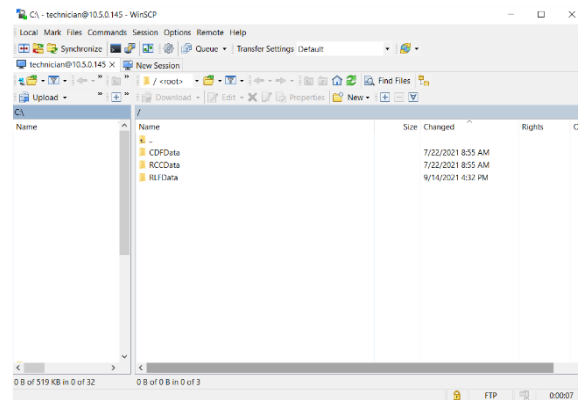
4. Copy and paste files from the RCCData folder to the personal computer.

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

Figure 104: The contents of the instrument server when using an FTPS client.
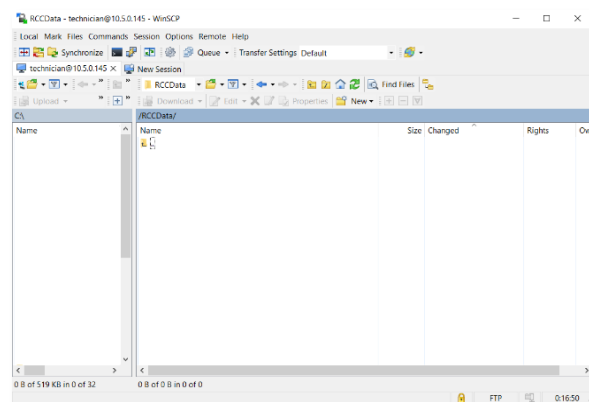
Figure 105: The contents of the 'RCCData' folder when using an FTPS client.

Technical Support & Maintenance

Technical Support

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

> **NanoString Technologies, Inc.**
>
> 530 Fairview Avenue North
>
> Seattle, Washington 98109 USA
>
> Website: https://www.nanostring.com/
>
> Support Email: support@nanostring.com
>
> Phone: +1 888 358 NANO (+1 888 358 6266)

Online Training at NanoString University

NanoString University is an online training platform for instructional videos and documents to accompany your NanoString instrument and assays. Create a free account at https://university.nanostring.com.

Prep Station Maintenance

Several tasks can be accomplished using the Maintenance Menu on the Prep Station:

- *system setup:* Set the date and time on the system clock and configure email.

- *hardware:* Home robot, align electrodes, lubricate O-rings and perform a dry run.

- *software:* Update Prep Station's system software when a new release occurs and view the current software information.

- *troubleshoot:* Download log files for remote analysis by NanoString Support.

- *shutdown system:* Power off the system.

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

The Maintenance Menu will appear (**Figure 107**).
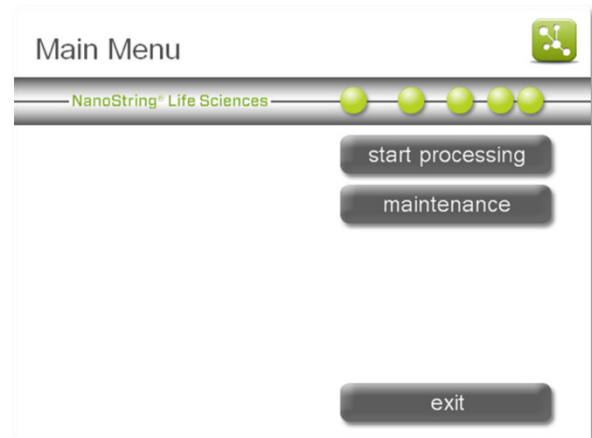
Figure 106: The Prep Station Main Menu.
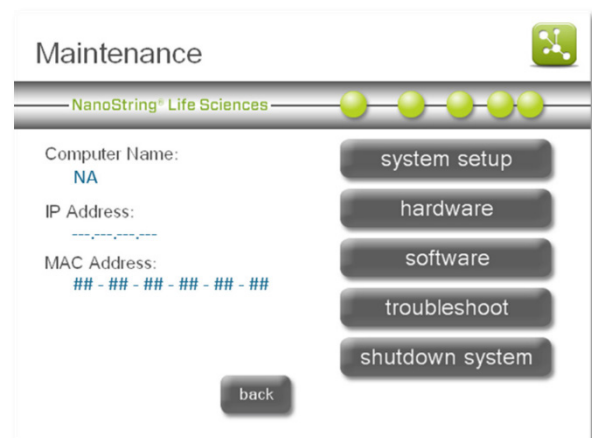
Figure 107: The Prep Station Maintenance Menu.

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System Setup

The System Setup Menu allows the user to set the Prep Station's date and time and configure email settings. To access it, press **system setup** on the Maintenance Menu (**Figure 107**).
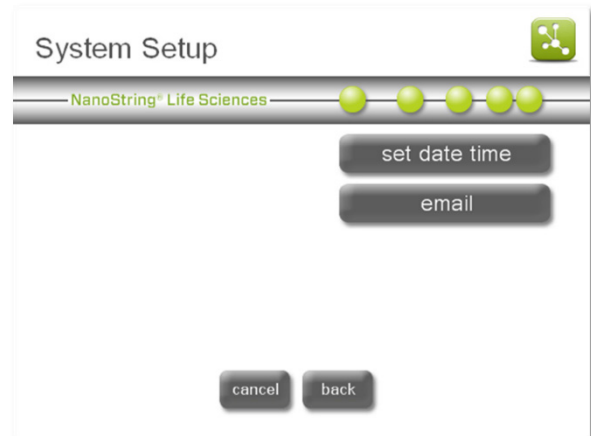
The System Setup Menu will appear (**Figure 108**).

Figure 108: The System Setup Menu.

Setting the System Clock

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 Savings Time.

Press **set date time**.

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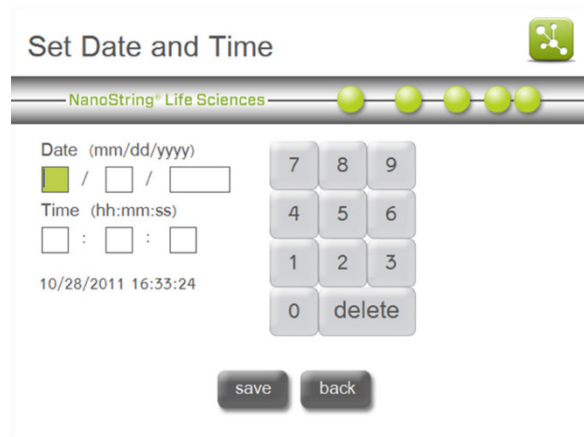
Figure 109: The 'Set Date and Time' screen.

Email Access

Email addresses can be added, deleted, and managed 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 email, press the **email** button on the System Setup Menu.

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

Figure 110: The 'Email configuration' screen.

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

There are several settings required to configure email 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.

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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**.
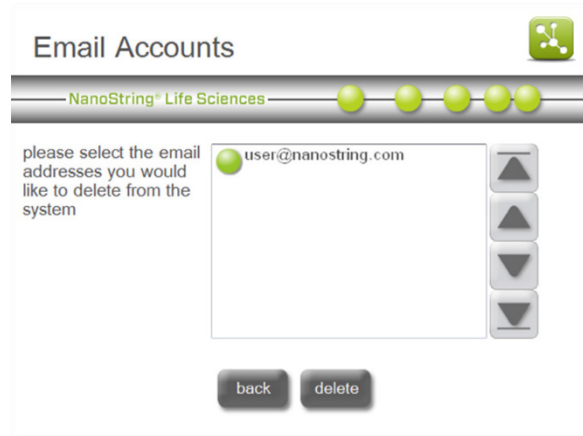

Figure 111: The 'Email Accounts' screen

System Hardware

To access the System Hardware Menu, press **hardware** on the Maintenance Menu. 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.

Figure 112: The System Hardware Menu.

- **calibration check:** NanoString recommends that customers do not run the calibration check program. If the Prep Station has been idle for more than 1 month, it is recommended to perform a *dry run*.

Home Robot

If the robot enters an undesirable state, pressing the **home robot** button can home all the motors. Pressing **home robot** will open the 'Home Prep Station' screen (**Figure 113**). This button should not be needed under normal operation.
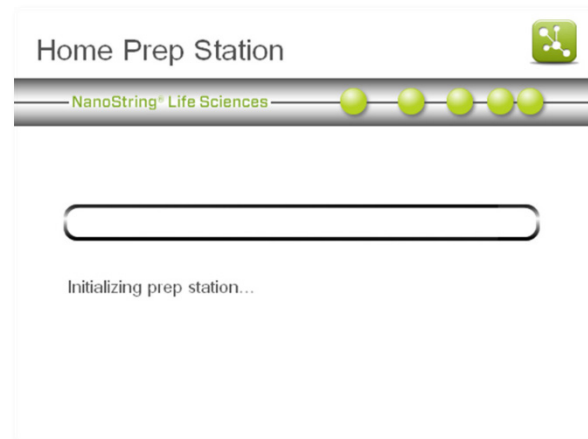

Figure 113: The 'Home Prep Station' screen.

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 contact the cartridge and bend.

> IMPORTANT: If the system software indicates a 'bent electrode' error message, contact support@nanostring.com before attempting to re-align an electrode. NanoString Support can assess whether the electrode is the true root cause of the error, reducing the risk of damage to your instrument.

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 close to 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 wells.

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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.

1. Select **align electrodes** on the System Hardware Menu.

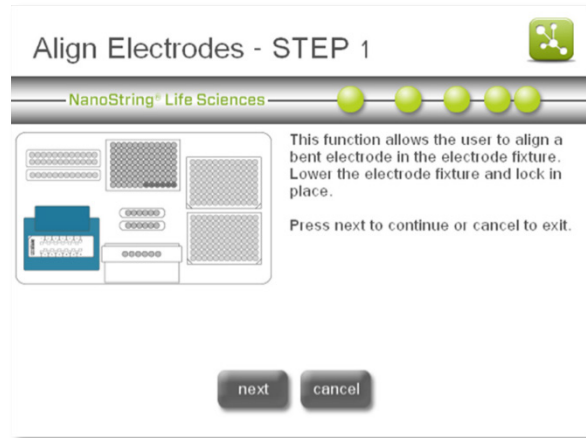   The 'Align Electrodes—STEP 1' screen will appear (**Figure 114**).

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

2. If the electrode is severely bent such that 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**).
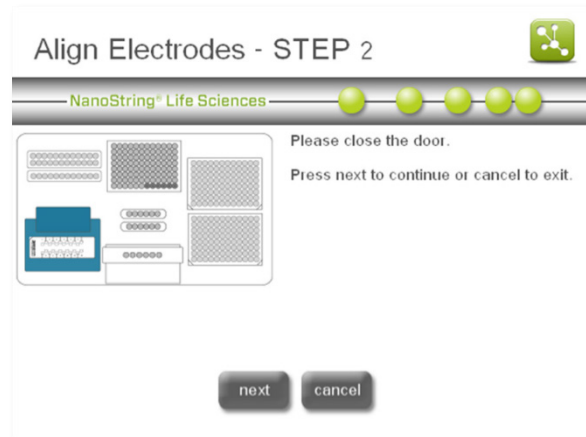
Figure 115: The 'Align Electrodes—STEP 2' screen.

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 116**).
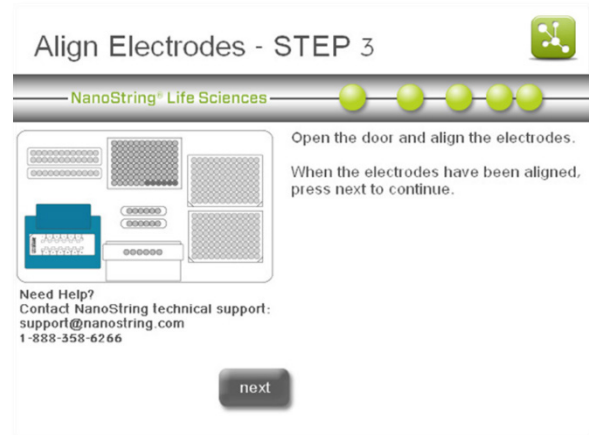
Figure 116: The 'Align Electrodes—STEP 3' screen.

3. 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**).
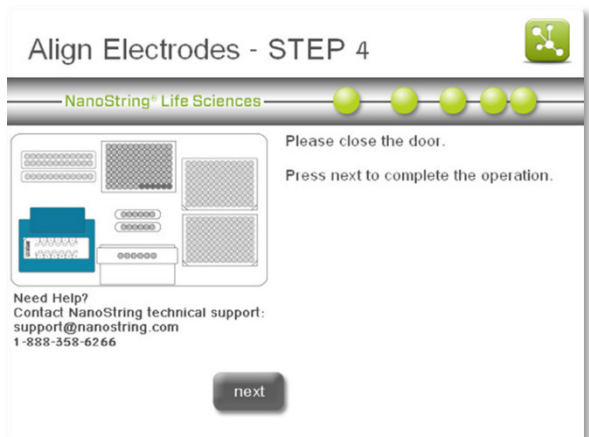
Figure 117: The 'Align Electrodes—STEP 4' screen.

4. 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**).

5. Press **finish** to return to the Maintenance Menu.
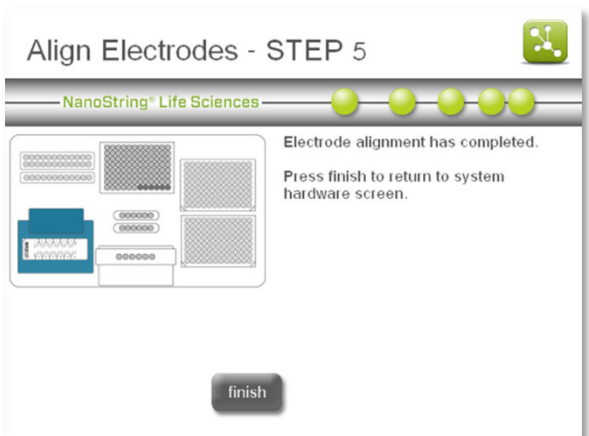
Figure 118: The 'Align Electrodes—STEP 5' screen.

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 (transparent grease in a plastic container, provided by NanoString)
- Lint-free paper (e.g., Kimwipe)
- Gloves
- Pipette tip

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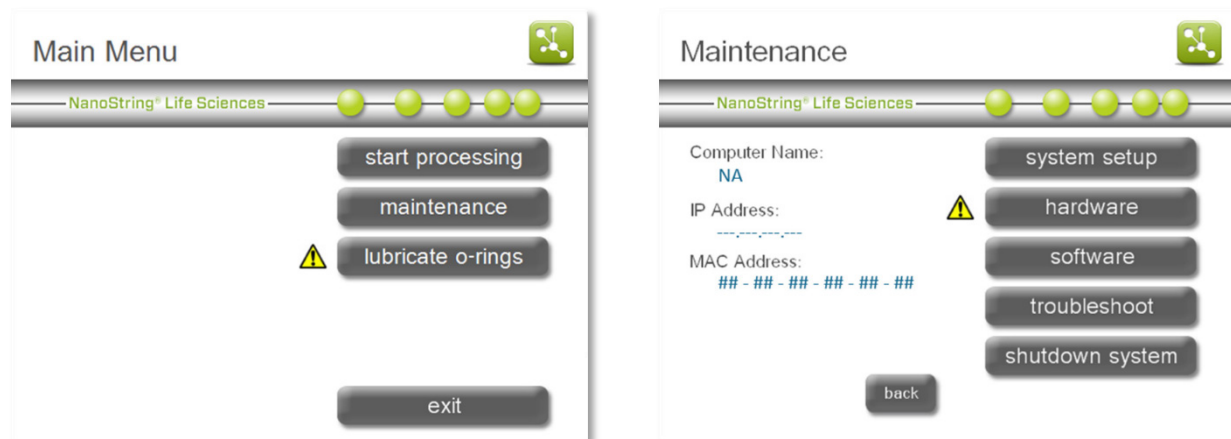
Figure 119: The 'lubricate O-rings' maintenance alert screens.

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

1. Select **lubricate O-rings** on the Main Menu or on the System Hardware Menu.

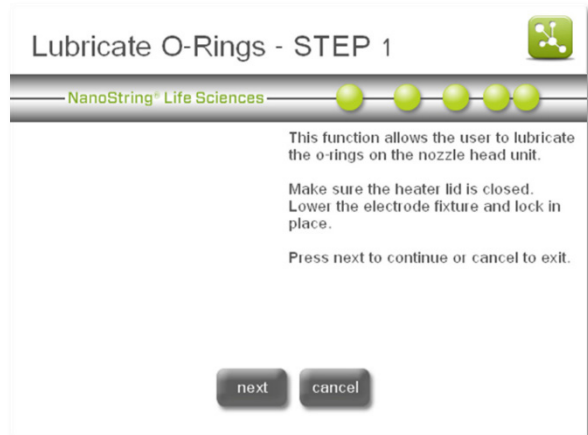   A series of 'Lubricate O-Rings' maintenance screens will appear (**Figure 120**).

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

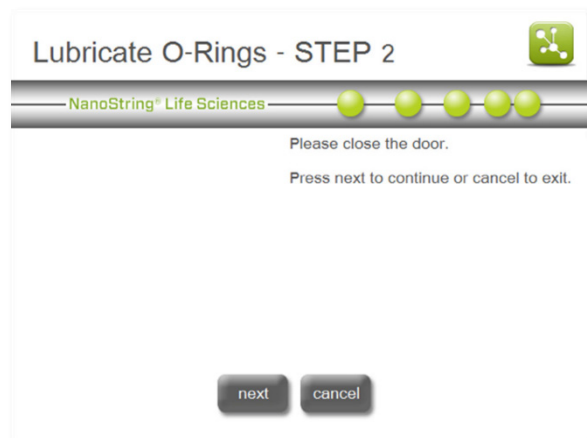2. Follow the instructions as directed on the screen (**Figure 121**).

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

3. To lubricate the O-rings, first use a twisted Kimwipe 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.

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Next, take a *very small* amount of silicone grease (1/3 of a grain of rice) and rub between gloved thumb and forefinger to yield a sheen of grease. Then, apply greased thumb and forefinger to the nozzle around the O-ring as shown (**Figure 122**).

After applying grease to the *front* and *back* of each nozzle, stick a pipette tip on the nozzle and twist it lightly back and forth, to spread the grease to the *sides* of the nozzles.

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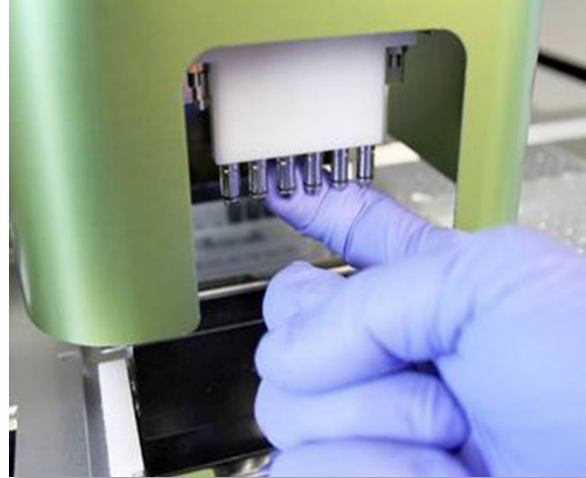
Figure 122: Use a finger to apply a small amount of NanoString silicone grease to the O-rings.

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 not to get grease *into* any nozzles. If grease does get in the nozzle, open up a paperclip and use the wire to carefully scrape the grease back out.

4. 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 such 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 re-lubricate 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.

4. When you get to the sample designation screen, process all twelve 'blank' samples. On the 'Select Protocol' screen, check the box to launch the Legacy Protocol (**Figure 124**).
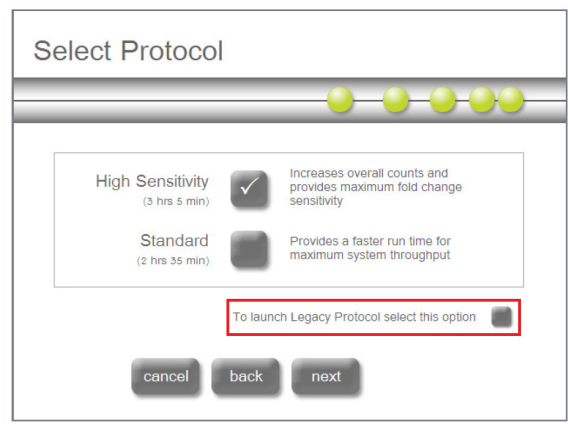

Figure 124: 'Select Protocol' screen

5. Approximately 10 minutes after initiating the dry run, the system will display 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 to stay with the Prep Station during the dry run, to correct any issues that may occur.

6. When the dry run is complete, 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 another dry run.

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 contact support@nanostring.com to create a support ticket and for guidance on how to submit the log files.
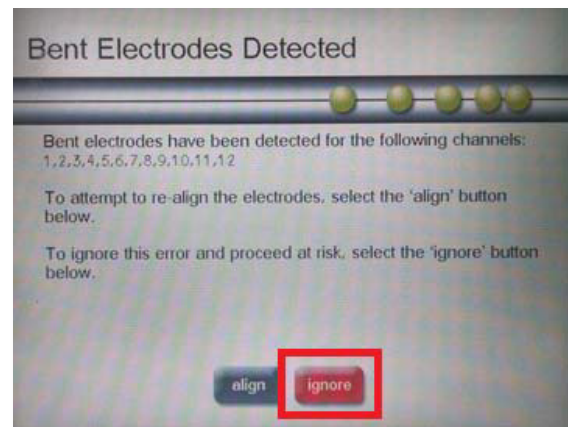
Figure 125: 'Bent Electrodes Detected' screen

System Software

To access the System Software Menu, press **software** on the Maintenance Menu. 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.
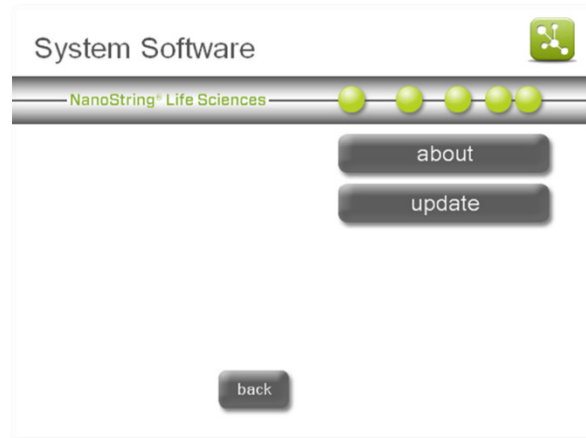
Figure 126: The System Software Menu.

About

If assistance with the Prep Station is required, please note the system software version prior to calling NanoString. This information can be found by pressing **about** on the System Software Menu to open the 'About' screen (**Figure 127**).

Figure 127: 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 website and transfer the update files to a USB flash drive. 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.

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Troubleshoot

To access the Troubleshoot Menu, press **troubleshoot** on the Maintenance Menu. The Troubleshoot Menu (**Figure 128**) displays options to download log files (to submit to NanoString Support for diagnosing instrument issues) or run specialized procedures that may assist in recovering failed runs.
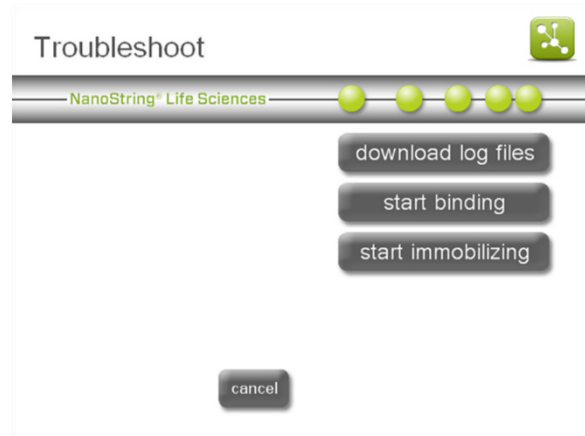
Figure 128: The Troubleshoot Menu.

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 pressing them (**Figure 129**). Selected folders will be highlighted in blue.

1. Insert a USB flash drive into the Prep Station's front USB port.

2. When the correct folders have been selected, press **download data** to transfer them to the USB flash drive.
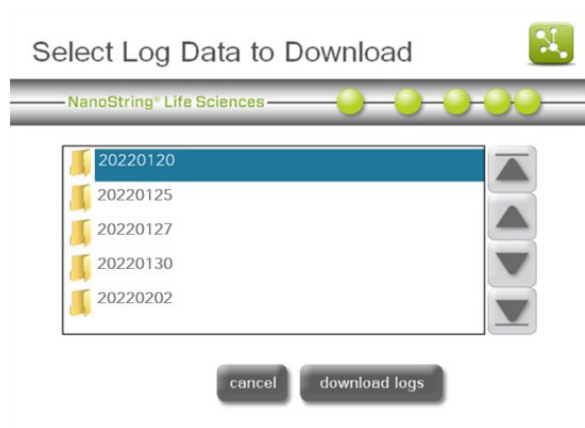
Figure 129: The 'Select Log Data to Download' screen.

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: 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.

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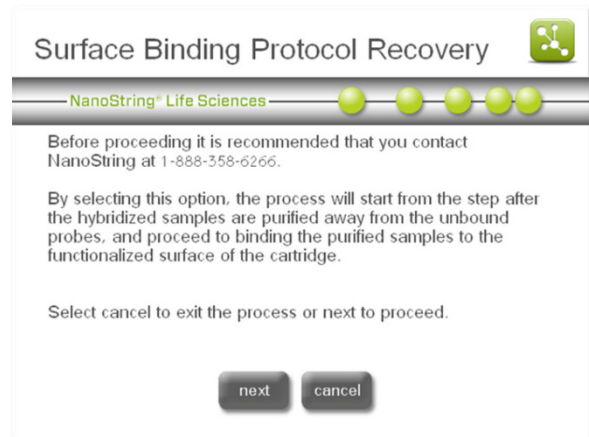
Figure 130: The 'Surface Binding Protocol Recovery' screen.

2. Instructions will appear on the next screen (**Figure 131**). Follow the instructions on the touchscreen to configure the system for the surface binding protocol.

    1) Purified samples will be dispensed, in order, in columns 7–12 of row D of the plate(s).

    2) Place prep plates on the deck.

    3) Load a new rack of tips.

    4) Load a sample cartridge.

    5) Place tip sheaths on the deck.

    6) Empty the waste bin and place it on the deck.

    7) Close the heater lid and electrode fixture.

    8) Press **next**.
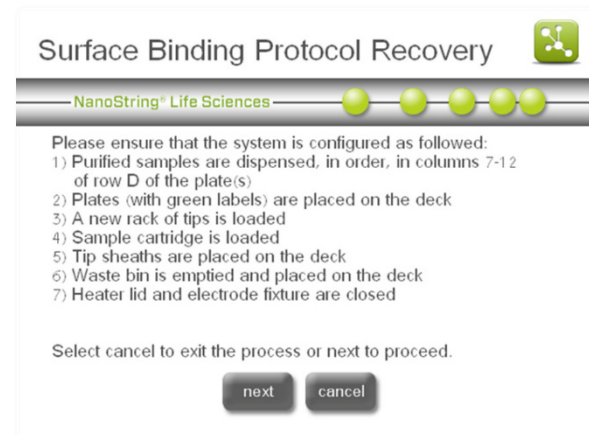
Figure 131: The 'Surface Binding Protocol Recovery' instructions.

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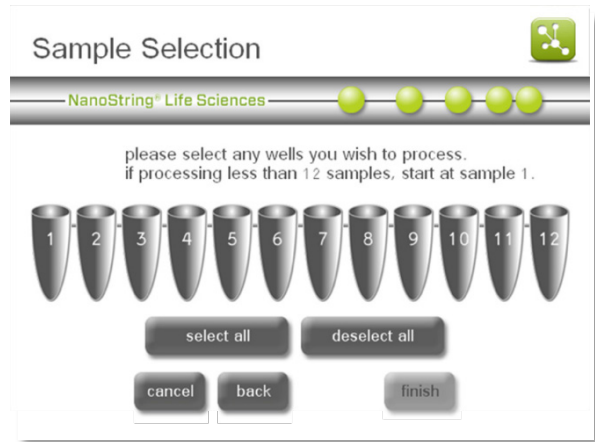
Figure 132: The 'Sample Selection' screen.

4. The 'Notification Options' screen will appear (**Figure 133**).

5. 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**.
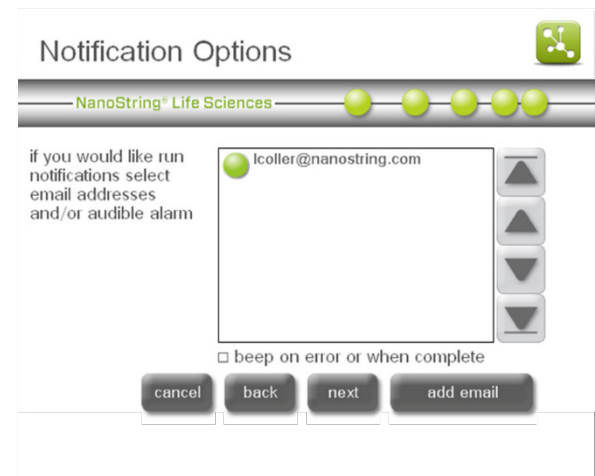

Figure 133: The 'Notification Options' screen

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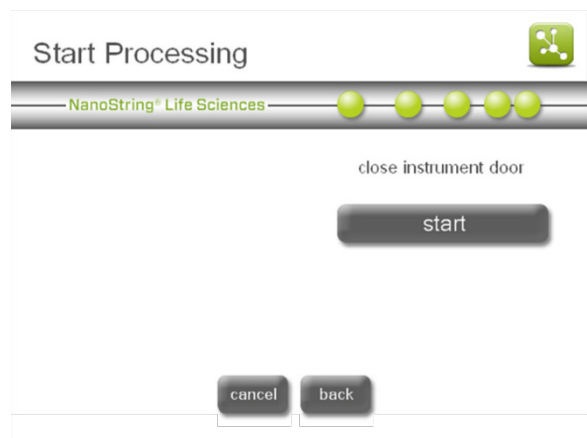
Figure 134: The 'Start Processing' screen.

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: Contact support@nanostring.com prior to starting surface immobilization.

1. To initiate the 'Surface Immobilization Protocol Recovery' workflow, press **start immobilizing** on the Troubleshoot Menu.

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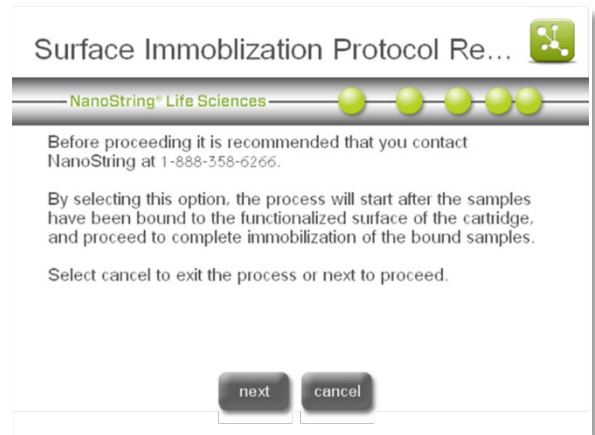
Figure 135: The 'Surface Immobilization Protocol Recovery' screen.

2. Instructions will appear on the next screen (**Figure 136**). Follow the instructions on the touchscreen to configure the system for the surface immobilization protocol.

   1) Confirm that the surface binding and cartridge buffer exchange are completed.

   2) Place prep plates on the deck.

   3) Load a new rack of tips.

   4) Load a sample cartridge.

   5) Place tip sheaths on the deck.

   6) Empty the waste bin and place it on the deck.

   7) Close the heater lid and electrode fixture.
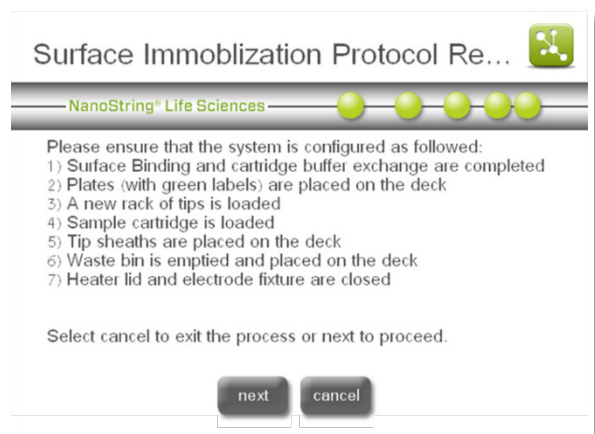
   8) Press **next**.

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

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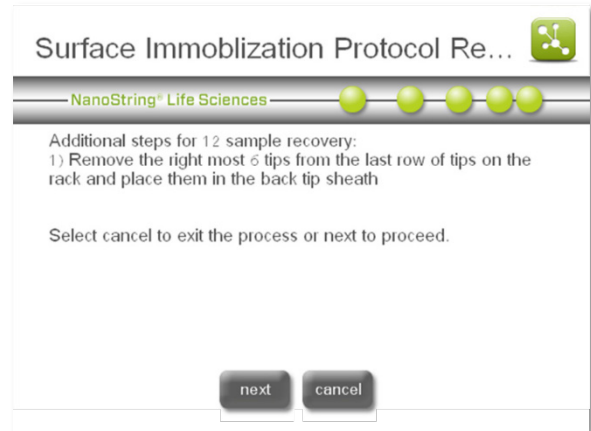

Figure 137: Additional instructions for the 'Surface Immobilization Protocol Recovery' workflow.

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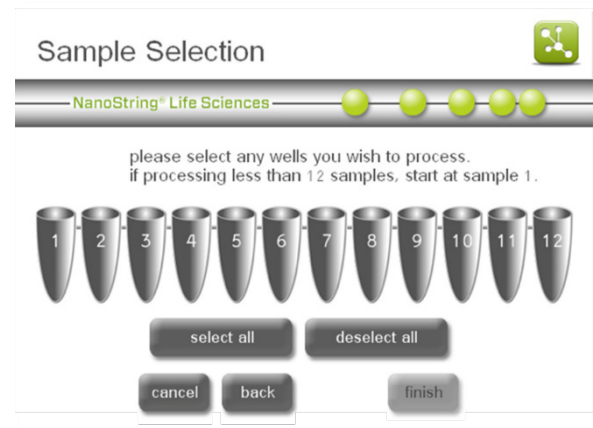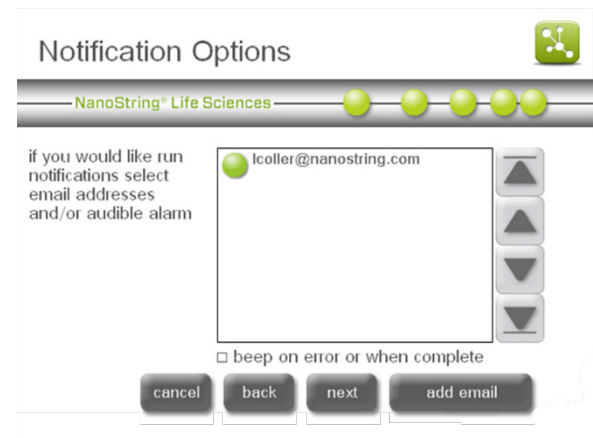

Figure 138: The 'Sample Selection' screen.

5. 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.

6. 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.
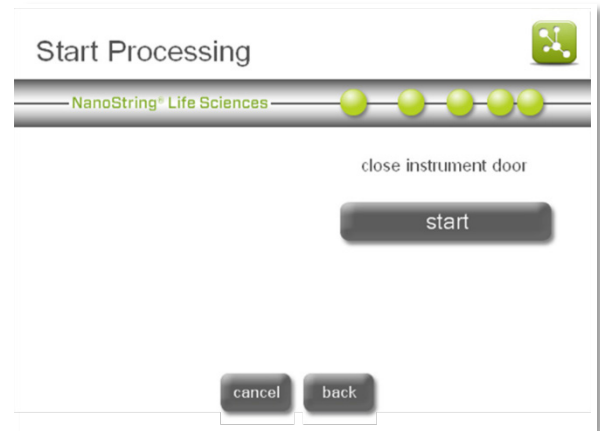
Figure 140: The 'Start Processing' screen.

Shutdown System

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

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Digital Analyzer Maintenance

Several tasks can be accomplished using the Maintenance Menu 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 email settings, and set the FTPS 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 Support.

- *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 flipping the power switch on the back of the instrument.

Each option is described in detail on the following pages.
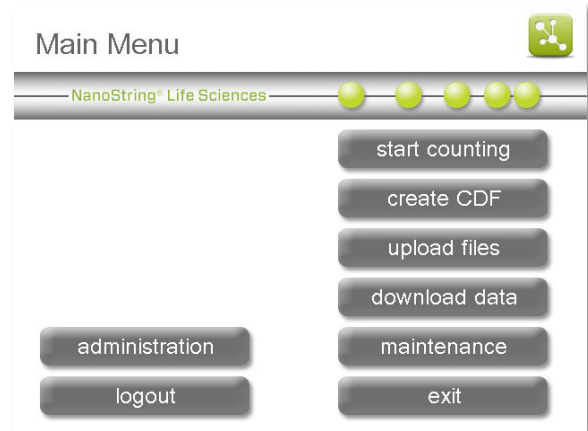
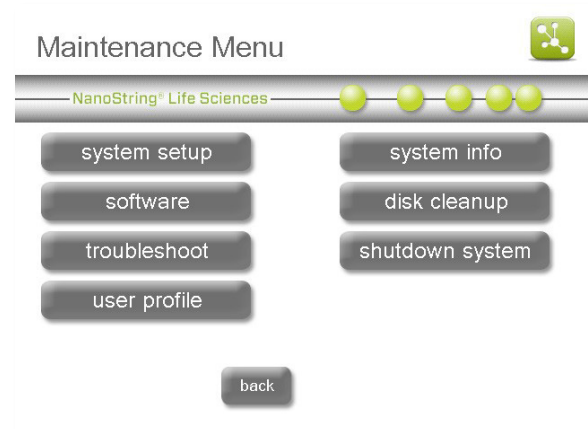

Figure 141: The Digital Analyzer Main Menu.

Figure 142: The Digital Analyzer Maintenance Menu.

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 email settings, and set the FTPS server password.
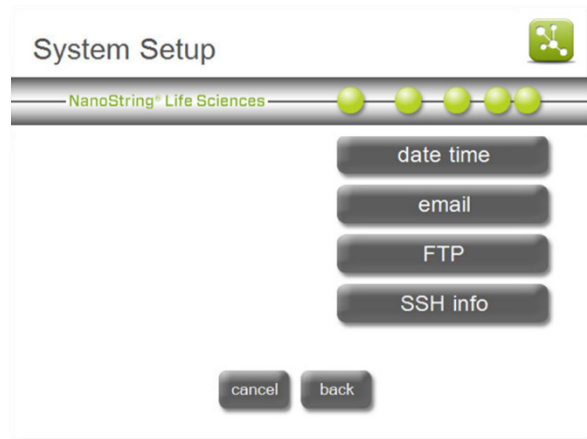

Figure 143: The 'System Setup' screen.

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**).
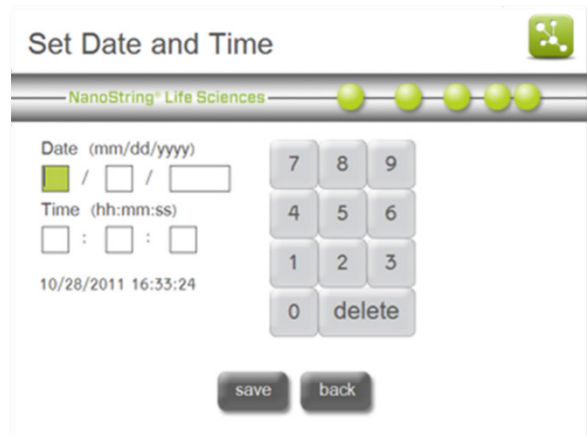

Figure 144: The 'Set Date and Time' screen.

Email Address

The system can send data to the user's email account if this option is configured (**Figure 145**). In the system screen, the user is only setting up the outgoing, or 'from', email 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 emailed to the account listed for each cartridge.

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

Figure 145: The 'Email configuration' screen.

There are several settings required to configure email 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.

FTPS Setup

1. To configure FTPS access to the Digital Analyzer, select **FTP** on the 'System Setup' screen. The 'FTP configuration' screen will appear (**Figure 147**). The default FTPS user name is set to "technician." The user name 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.

3. To disable FTPS access to the Digital Analyzer, press the **enabled** field so that it displays "no (disabled)", and no FTPS access will be allowed.
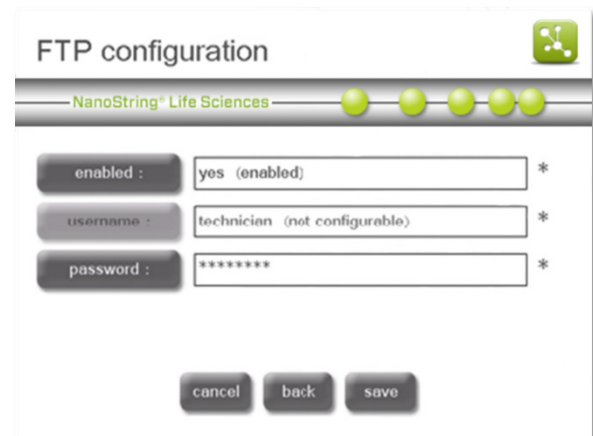
Figure 147: The 'FTP configuration' screen.

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

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SSH (SFTP) Setup

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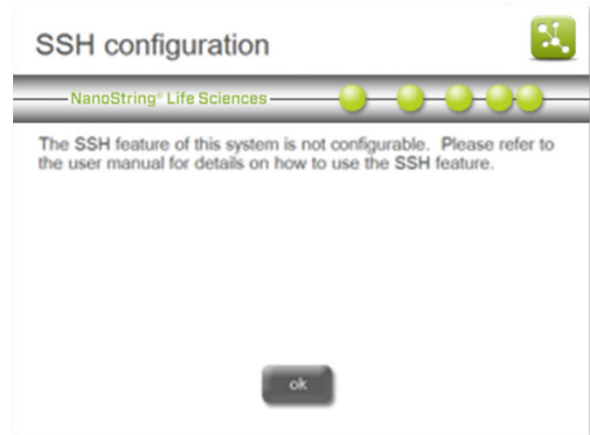

Figure 148: The 'SSH configuration' screen.

System Software

Select **software** from the Maintenance Menu 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**).

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**).
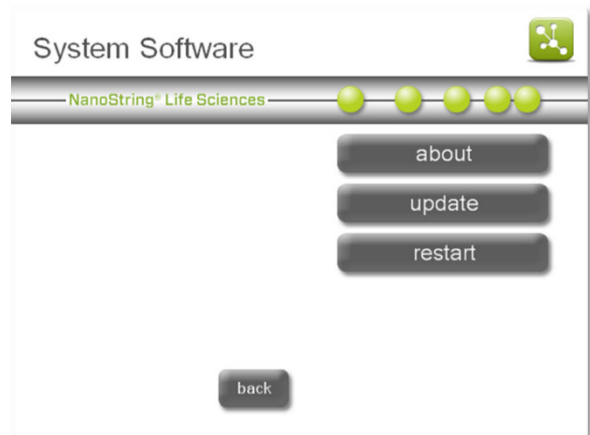
Figure 149: The 'System Software' screen.

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 website and transfer the update files to a USB flash drive. The update file (*.cab) needs to be at the root directory of the USB flash drive. 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.

Figure 150: The 'About' screen.

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 Support for assistance in diagnosing a problem.

1. 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.

4. 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.
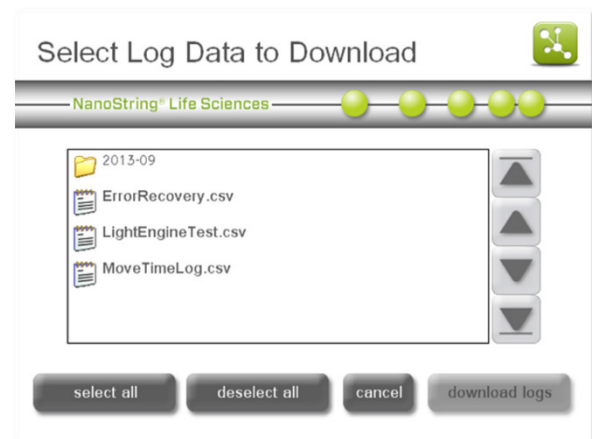
Figure 152: The 'Select Log Data to Download' screen.

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. Contact support@nanostring.com to open a support ticket and for guidance on submitting the log files to NanoString Support. A representative will review the log files to help determine the cause of any issues.

Figure 153: The 'Successful download' message screen

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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
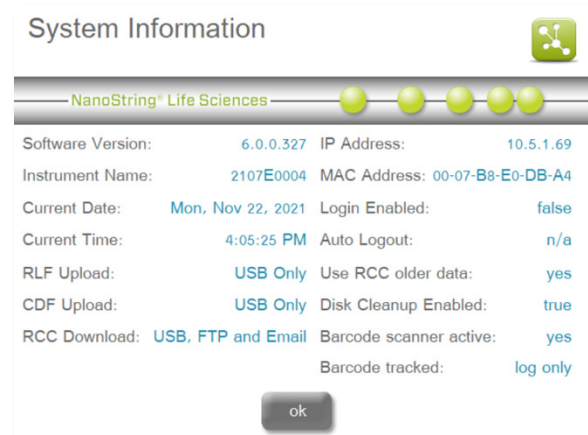
Figure 154: The 'System Information' screen.

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 to check the current disk space usage (used and free), to delete RCC files, CDFs, and 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.
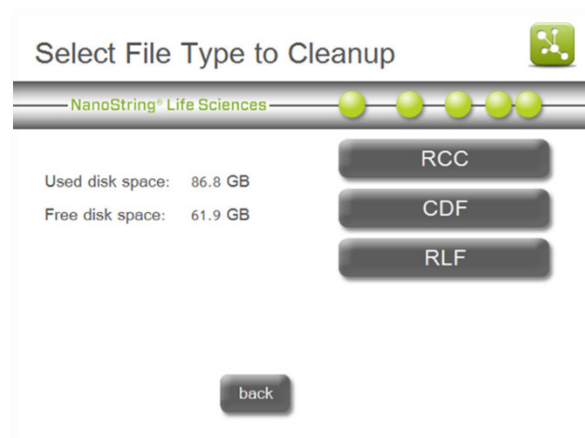
Figure 155: The 'Disk Cleanup' screen.

Cleanup RCC Files

1. 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**.

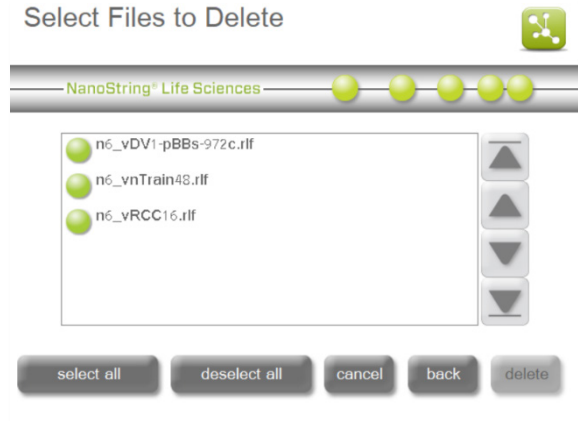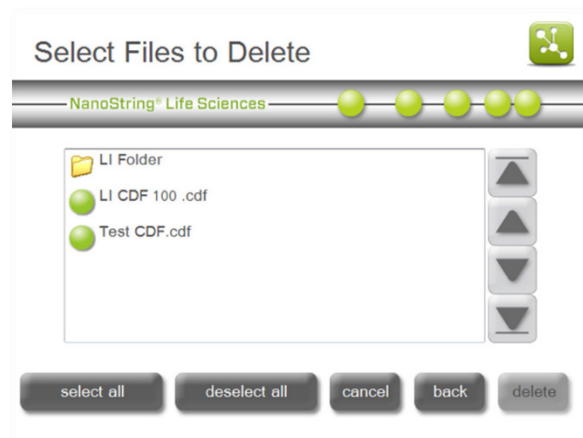3. At the prompt, confirm the files to delete by pressing **yes**.

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

Cleanup CDFs

1. 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**.

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

Cleanup RLFs

1. 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**.
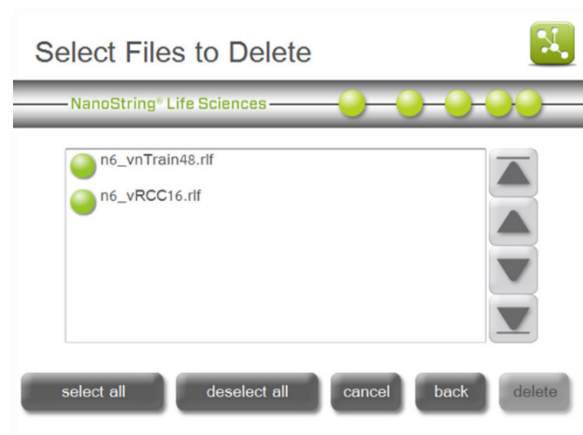
Figure 158: The 'Select Files to Delete' screen.

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Shutdown System

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

Cleaning

After a completed Prep Station 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 RNase*Zap*® from Ambion® may also be used.

Clean instruments' 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 symbol)	Manufacturer
EC | REP	Authorized Representative in the European Community (Safety)
CE	Safety CE Mark
UKCA	Safety UK Conformity Assessed Mark
REF	Catalogue or Reference Number
LOT	Batch code / Lot number
SN	Serial number
(Temperature range symbol)	Temperature range storage conditions
(Lower limit temperature symbol)	Lower limit of temperature storage conditions
(Upper limit temperature symbol)	Upper limit of temperature storage conditions
(Hourglass symbol)	For Use by / Expiry Date
(Date of manufacture symbol)	Date of Manufacture (DOM)

Regulatory Disclaimer

For applications for which approval/clearance is not required and research use.

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