

nCounter[®]

Digital Analyzer User Manual (GEN2)

NanoString Technologies, Inc.

530 Fairview Ave N
Suite 2000
Seattle, Washington 98109

www.nanostring.com

Tel: 206.378.6266
888.358.6266

E-mail: info@nanostring.com

Molecules That Count[®]

Translational Research • Gene Expression • miRNA Expression • Epigenomics • Copy Number Variation

MAN-C0021-01

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Original Instructions

This manual contains the original instructions for the nCounter® Digital Analyzer and is the native language version of the manual.

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PREFACE

Purpose



This user manual details specific information pertaining to safe and proper handling, operation and maintenance of the nCounter® Digital Analyzer.

Conventions Used

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

Note Types

Special font formatting is used in this manual. Such formatting conventions are used in specific instances as described below:

TIP		Information contained in a Tip may offer helpful suggestions, alternative procedures, methods and/or shortcuts.
NOTE		This note type emphasizes general information.
IMPORTANT		This note type presents essential content indicating that the potential exists for assay failure, diminished data quality, and/or a loss of data if the information presented is ignored.
WARNING		This note type indicates that a potential hazard to your personal safety, or the potential for equipment damage exists.
BOLD		When appearing in text or in a procedure, the bold text serves to highlight a specific button, key stroke, or menu option available. <ul style="list-style-type: none">• Bold text may appear elsewhere to highlight important text or terms.• Green text is used to help the reader identify active hyperlinks.
ITALICS		Used to emphasize an important word or expression within the text. <ul style="list-style-type: none">• Formatting of a book title, journal, or other documentation.• Used to indicate the special or unusual meaning of a word or phrase.

Procedures

Numbered procedures appear frequently providing step-by-step instruction for accomplishing a task. Typically, a numbered step provides direction for a specific action and may be followed by the expected response. Additional information may be presented in the form of a specific note type, bullets, screen capture or other image important to facilitate clarity and understanding. For example:

In the (next) screen, the active data entry field is indicated by a green box around it. Simply move from one field to the next, simply press the desired field on the touchscreen with your finger.

1. To add an email address, press **ADD**.
>>> The email address keyboard screen appears.
2. Enter a valid email address and press **ENTER**. The email address gets saved.

Contact Information

NanoString Technologies, Inc.

530 Fairview Ave N
Suite 2000
Seattle, Washington 98109 USA

Tel: 206.378.6266
888.358.NANO (6266)
Fax: 206.378.6288
Email: support@nanosttring.com

1 General Information

This chapter provides a general introduction to the instrumentation of the GEN2 nCounter System as well as specific information pertaining to the nCounter Digital Analyzer. Specific information provided in this chapter includes:

- Serial Number Location
- Safety Considerations
- Specifications
- Installation Information and Environmental Conditions
- Maintenance, Service and Support

nCounter® Analysis System (GEN2)

The GEN2 nCounter Analysis System is comprised of two instruments, the Prep Station used for post-hybridization processing, and the Digital Analyzer used for data collection.

NOTE: GEN2 Prep Stations and Digital Analyzers began shipping in November 2011, and can be identified by the “5s” designation on the name plate located on the back of the instruments.

NOTE: The Prep Station and the Digital Analyzer are stand alone units. These instruments do not require connection to an external PC and are not networked to one another. They may be set up and installed separately. Connecting the Digital Analyzer to a network is optional, and this enables data delivery to the user via a network connection. Likewise, if the Prep Station is networked, then notifications can optionally be sent to users.

The GEN2 nCounter Analysis System uses Setup and Process wizards on an embedded touchscreen user interface to guide you through the sample processing and data collection steps of the assay. Following the instructions will walk you through setting up runs step-by-step on the Prep Station and Digital Analyzer.

nCounter® Prep Station Overview

The nCounter Prep Station is the automated fluidic handling component of the GEN2 nCounter System and processes samples post-hybridization to prepare them for data collection on the nCounter Digital Analyzer.

FIGURE 1.1: nCounter Prep Station



Prior to placing samples in the Prep Station, samples need to be hybridized according to the protocol outlined in The nCounter Gene Expression Assay Manual, The nCounter miRNA Assay Manual and the nCounter CNV Assay Manual. On the deck of the Prep Station, hybridized samples are purified and immobilized in a Sample Cartridge for data collection.

FIGURE 1.2: nCounter Cartridge



All consumable components and reagents required for sample processing on the Prep Station are provided in the nCounter Master Kit and are ready to load onto the deck of the robot. No reagent preparation or dilutions are required. The Prep Station can process up to 12 samples per run in less than two and a half hours.

nCounter® Digital Analyzer Overview

The nCounter 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 highest standard data resolution, 280 fields of view (FOV) are collected per flow cell (sample) yielding data of hundreds of thousands of target molecule counts. The number of images taken corresponds to the number of reporters counted and this, in part, determines the dynamic range and level of sensitivity in the system.

Images are processed internally and the results are exported as a comma separated values format file that can be downloaded via memory stick. The file can be opened by most commonly used spreadsheet packages, including Microsoft® Excel and can be analyzed using NanoString's nSolver Analysis Software or other data analysis and visualization software packages.

FIGURE 1.3: nCounter Digital Analyzer



Touchscreen Overview

The nCounter System's touchscreen is a pressure-sensitive method of controlling the Prep Station and Digital Analyzer which enables the user to interact with the system by touching a selection on the screen. There are several standard option 'buttons' that appear in the touchscreen user interface ([Table 1.1](#)).

TABLE 1.1: nCounter system's touchscreen interface buttons

Button	Action
Next	Proceeds to the next screen.
Back	Moves back to the previous screen.
Cancel	Returns to beginning of the current workflow or the main menu.
Save	Saves entered data, and allows you to continue. The Save button is primarily used during file creation, upload, and download.
Done	Allows you to skip instructional steps and jumps to the next screen that requires input. The Done button is also used to indicate that data entry is complete and that you are ready to initiate processing.

nCounter Digital Analyzer Information

For proper operation and testing results, the instructions in this manual must be followed by experienced personnel. It is important to read and understand the contents of this manual prior to operating the GEN2 nCounter Analysis System. For service and support information, please refer to **General Maintenance, Service and Support on page 7**.

NanoString Technologies does not assume any liability arising from your use of the GEN2 nCounter Analysis System, this manual or other documentation provided by NanoString.

Serial Number

Each nCounter Digital Analyzer has a serial number located on the back panel of the unit next to the power plug. Please include your serial number in communications with NanoString pertaining to the Digital Analyzer.

Serial Number _____

Date Received _____

Specifications

Description	Specifications
Power supply	100–240 VAC, 50–60 Hz, 610 VA
Size (W x D x H)	26 x 26 x 19 inches; 66 x 66 x 48 cm
Weight	150 lbs; 68 kg

Important Safety Information

Please read through this manual and the following guidelines carefully to ensure safe operation of the NanoString nCounter Digital Analyzer. Always keep this manual near the instrument 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 will void the manufacturer's warranty.

In the event of an Emergency, immediately turn the power switch OFF and unplug the Digital Analyzer.



WARNING: Ensure that water or chemicals do not come in contact with any part of the equipment. Water or chemicals may cause damage and will void the warranty.



IMPORTANT: Place the instrument in a location where the on/off switch is readily accessible.



NOTE: Unplug the equipment when not in use for extended periods of time.



NOTE: When using the equipment, follow generally accepted procedures for quality control.



WARNING: Do not attempt to disassemble the equipment. The nCounter Digital Analyzer contains no user-serviceable parts. Service personnel trained by the manufacturer must perform repairs. Do not modify any part of the equipment as this may cause fire, malfunction, and will void the manufacturer's warranty.

The (NanoString) Digital Analyzer is equipped with an internal laser barcode reader. The laser complies with US 21CFR1040.10 and 1040.11 except for deviations pursuant to Laser Notice No. 50 dated July 26, 2002 and IEC60825-1:2007.

Always take note of laser safety labels; they indicate areas where exposure to laser beams may be hazardous. When used according to the instructions in this manual and when all covers are in place, the (NanoString) Digital Analyzer is classified as a Class 1 Laser Device per 21 CFR 1040 and IEC/EN 60825-1.



WARNING: The (Nanostring) Digital Analyzer is a Class 1 laser product. The Digital Analyzer contains an internal Class 2 laser barcode reader. Class 2 laser radiation exposure if the Digital Analyzer cover is opened. Do not stare into barcode laser reader beam.



WARNING: Use the Digital Analyzer only as instructed in this user guide. Do not attempt to service the instrument. Only qualified service technicians can open and service the Digital Analyzer. There are no customer serviceable parts. Removing the case exposes the customer to laser and electrical shock hazards.



NOTE: Disposal of Electronic Equipment

It is important to understand and follow all laws regarding the safe and proper disposal of electrical instrumentation. The symbol of a crossed-out wheeled bin on the product 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.

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.


Safety Compliance

The Digital Analyzer was developed, manufactured and tested in accordance with all applicable national and international safety standards and is listed with TÜV, a Nationally Recognized Testing Laboratory.



FIGURE 1.4: Declaration of Conformity

CE



Declaration of Conformity

in accordance with EN ISO 17050-1-2004

Manufacturer:	NanoString Technologies, Inc.
Address:	530 Fairview Avenue N Suite 2000 Seattle, WA 98109
Telephone:	(206) 378-6266

The undersigned hereby declares, on behalf of NanoString Technologies of Seattle, WA, that the following Product conforms to all the relevant provisions of the Directives listed.

Product:	Epifluorescence Microscope
Model Name:	nCounter Digital Analyzer 5s
Council Directives	
Machinery:	2006/42/EC
EMC:	2004/108/EC
Applicable Standards	
Safety:	UL 61010-1/R:2008-10 IEC 61010-1:2001 IEC 61010-2-101:2002
EMC:	EN 61326-1:2006 EN 61326-2-6:2006
Serial Number:	XXXXCYYYY

Place _____

Date _____

Name _____

Signature _____

Position _____

The technical documentation for the Product is available from:

Name:	Derek Graeme Potter
Address:	9 Grange Gardens Banstead SM7 3RF United Kingdom

Declaration of Conformity

NanoString Technologies

Installation and Environmental Conditions

The following table provides information on the environmental requirements necessary for the safe operation of the nCounter Digital Analyzer. Installation and dismantling of the Digital Analyzer must be performed by a qualified representative of NanoString Technologies.

TABLE 1.2: Environmental conditions required for the nCounter Digital Analyzer

Installation site	<ul style="list-style-type: none"> Indoor use only. The Digital Analyzer should be placed on flat, stable surface with access to an electrical power source (see power source requirements below). Avoid locating the Digital Analyzer near large electrical equipment to prevent possible interference from noise and/or voltage fluctuation. Avoid locating in direct sunlight. Avoid locating where the potential for vibration from other sources exists. Avoid locating in an excessively dusty environment. If connecting the Digital Analyzer to a network, locate the instrument in an area with access to a network cable.
Power source	<ul style="list-style-type: none"> An independent power outlet used only for the nCounter Digital Analyzer is required. The Digital Analyzer requires an electrical power source at 100-240 VAC, 610 VA and 50/60Hz. Always use a grounded power outlet and use the power cable provided by NanoString in order to ensure proper grounding. The use of a power surge protector and an uninterrupted power supply (UPS) is highly recommended.
Temperature	<ul style="list-style-type: none"> 18 to 28°C
Humidity	<ul style="list-style-type: none"> 30 to 80% relative humidity (non-condensing)

Storage and Transport

Conditions should comply with ISTA2B standards, which are 5°C - 38°C, 0-85% RH (non-condensing).

General Maintenance, Service and Support

Cleaning

When cleaning the nCounter Digital Analyzer, it is important to follow all safety and operating instructions provided in this manual. Always use safe laboratory operating precautions, including wearing safety glasses and gloves.

Guidelines

- Always keep the lid of the Digital Analyzer closed when not in use to minimize dust.



IMPORTANT: DO NOT attempt to clean the objective lens.

Technical Support

Technical support is available by phone, fax, mail, or email. Please be sure to include your serial number in communications. Please refer to **Serial Number on page 10** for information on locating the serial number of your product.

NanoString Technologies, Inc.

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Suite 2000
Seattle, Washington 98109 USA

Tel: 206.378.6266
888.358.NANO (6266)
Fax: 206.378.6288
Email: support@nanosttring.com

Purchasing

All reagents and consumables required to process 48 or 192 nCounter Gene Expression Assays are supplied in the nCounter Master Kit (individual kit components may be purchased separately if required). For further information, please refer to **Consumables Required section of the GEN2 nCounter Prep Station User Manual**.

Accessories and consumables may be purchased from NanoString Technologies. Please contact your representative directly, or email sales@nanosttring.com to have a representative contact you.

2 Running the nCounter Digital Analyzer

Introduction

nCounter® Assays are designed to provide an ultra-sensitive, reproducible and highly multiplexed method for detecting nucleic acid species across all levels of biological expression. This assay provides a method for direct detection of specific nucleic acid sequences with molecular barcodes called nCounter Reporter Probes without the use of reverse transcription or amplification.

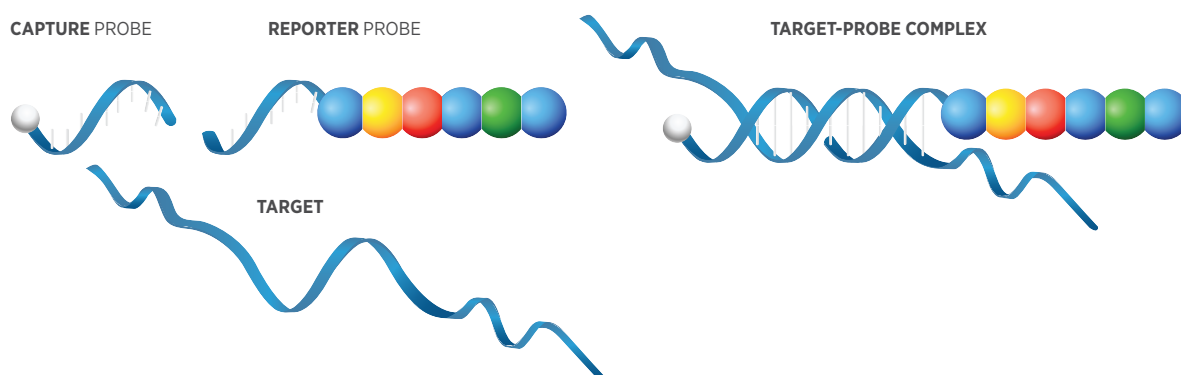
This manual describes the process for collecting data from your assay using your nCounter Digital Analyzer. Please refer to the nCounter Gene Expression Assay Manual, nCounter miRNA Assay Manual, nCounter CNV Assay Manual and nCounter Prep Station User Manual, for information on setting up your assay and post- hybridization processing. Please refer to the nSolver Analysis Software Manual and assay specific data analysis guides for information on data analysis.

NanoString Technology

Principles and Procedures

NanoString's technology is based on digital detection and direct molecular barcoding of target molecules through the use of a color coded probe pair. The probe pair consists of a Reporter Probe, which carries the signal on its 5' end, and a Capture Probe which carries a biotin on the 3' end. The color codes carry six positions and each position can be one of four colors, thus allowing for a large diversity of tags that can be mixed together in a single well for direct hybridization to target and yet still be individually resolved and identified during data collection.

FIGURE 2.1: Capture and Reporter Probes (left) and, Probe pair bound to an mRNA (right)



Probe pairs are placed into a reaction in massive excess to target RNA or DNA species to ensure that each target finds a probe pair. After hybridization, excess probes are washed away using a two step magnetic bead-based purification on the nCounter Prep Station.

Magnetic beads derivatized with short nucleic acid sequences that are complementary to the Capture Probe and the Reporter Probes are used sequentially. First, the hybridization mixture is allowed to bind to the magnetic beads by the Capture Probe. Wash steps are performed and excess Reporter Probes and non-target cellular transcripts are removed during wash steps. After washing, the Capture Probes and Target/Probe complexes are eluted off of the beads and are hybridized to magnetic beads complementary to the Reporter Probe. Wash steps are performed and excess Capture Probes are washed away. Finally, the purified Target/Probe complexes are eluted off and are immobilized in the cartridge for data collection.

Data Collection is carried out in the nCounter Digital Analyzer. At the highest standard data resolution, 280 fields of view (FOV) are collected per flow cell (sample) using a microscope objective and a CCD camera yielding data of hundreds of thousands of target molecule counts. Digital images are processed on the nCounter Digital Analyzer and the barcode counts are tabulated in a comma separated value (CSV) format.

The nCounter Analysis System was created by NanoString Technologies. The nCounter system is an easy-to-use integrated system that includes a Prep Station (robot) and a Digital Analyzer (analyzer). The Prep Station and the Digital Analyzer together make lab work and sample analysis a simpler process by limiting the variables in experiments for lab technicians. The end result is a very precise and accurate measurement, enabling you to gather data on your targets of interest rapidly with minimal intervention.

nCounter® Expression Assay Overview

The nCounter Expression Assay is run on the nCounter System. The system is comprised of two instruments, the nCounter Prep Station used for post-hybridization processing, and the Digital Analyzer used for data collection. Follow the instructions on the touchscreen to guide you step-by-step through setting up runs on the Prep Station and Digital Analyzer. For further details on the nCounter System instrumentation, please refer to [The nCounter Analysis System on page 1](#).

FIGURE 2.2: Suggested workflow for the nCounter Expression Assay

Day 1	Manual Processing	Hands-on Time
	Set Up Hybridization	5 minutes
Day 2	Automated Processing	Hands-on Time
	Set Up Prep Station Run	5 minutes
	Set Up Data Collection	5 minutes

Overview of File/Folder Structure

There are three file types used by the nCounter Analysis System; the Cartridge Definition File (CDF) the Reporter Library File (RLF) and a Reporter Code Count (RCC) file. A Cartridge Definition File is created by the user and defines sample specific data to associate with the data output and defines the parameters for the imaging instrument to use during image collection & processing. The Reporter Library File is generated by NanoString and it is unique to your custom CodeSet. The RLF 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 and contains the data for one flow cell detailing the number of counts for each target.

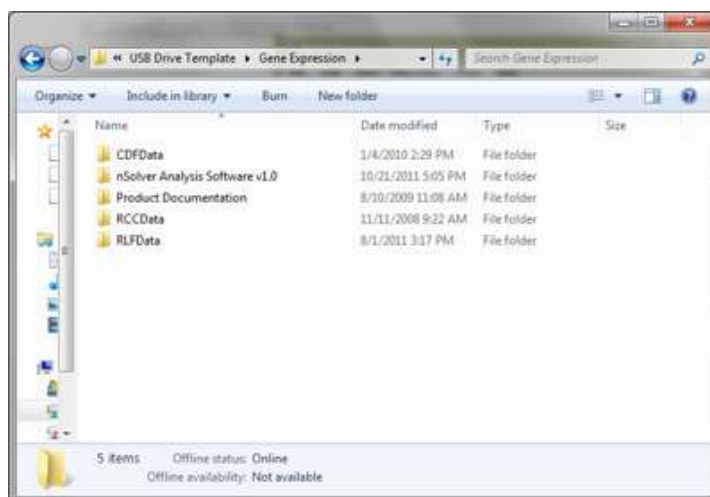
For use on the nCounter Digital Analyzer, each custom CodeSet ships with a memory stick containing the following:

- Three read-only folders for Cartridge Definition files (CDFData), Reporter Library files (RLFData), and Reporter Code Count files (RCCData) (**Figure 2.3**)
- A CDF template
- A compressed file containing the nSolver Analysis Software installer for Windows®, a PDF copy of the nSolver Analysis Software Manual and a folder containing sample RCC files.
- A compressed file containing the nSolver Analysis Software installer for Macintosh®, a PDF copy of the nSolver Analysis Software Manual and a folder containing sample RCC files.
- Product specific assay manuals, product inserts, and data analysis guides in PDF format.
- The nCounter Prep Station User Manual and the nCounter Digital Analyzer User Manual
- Material Safety Data Sheet(s)

You may use your own memory sticks to transfer files but you must create the same three folders on your personal memory stick in order to interface with the system.

NOTE: The memory stick and the Digital Analyzer have been designed specifically to enable quick and accurate file transfers. You may create sub-folders for CDFs on the memory stick to facilitate file organization. However, you should not create sub-folders for RLFs or RCC files. If you set up sub-folders within the RLF folder on your memory stick, the system will not be able to locate the RLF files for transfer.

FIGURE 2.3: Memory stick file folders



Navigating File/Folder Windows

The file/folder structure is set up similar to Windows with nested folder structures. The root, or top level, directory is indicated by the two periods before the folder name. For example, “..(CDFData)” as shown in **Figure 2.4**.

From there, navigate down in folder structures by “double-touching” the folder to open it like you normally would with a mouse double-click (Figure 2.4). To navigate back to a higher level folder, simply double-touch the root directory and you will be back at 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 that have lines at the top or bottom to go to the very top or the very bottom of the file list.

FIGURE 2.4: Navigating files/folders from the Digital Analyzer screen

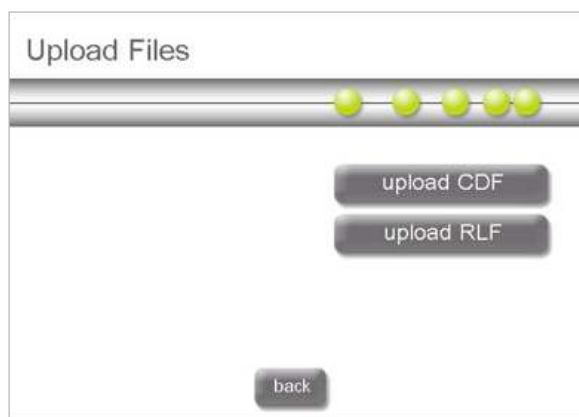


Uploading Reporter Library Files

You only need to upload RLF files when you first receive a new CodeSet. Once an RLF has been uploaded, it will always be available until it is manually deleted from the Digital Analyzer. You will need a pre-loaded RLF corresponding to your CodeSet when you set up a run using that CodeSet. Instructions for deleting RLF files are found in the Maintenance chapter (refer to [Chapter 3, Maintenance and System Setup on page 41](#)).

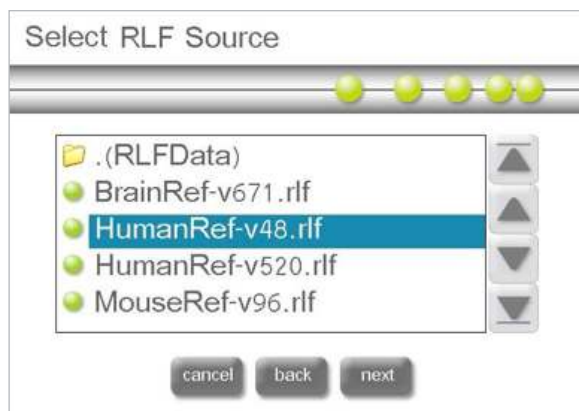
1. Insert the memory stick.
2. From the **main menu**, press **Upload Files**.
>>> The Upload Files screen appears ([Figure 2.5](#)).

FIGURE 2.5: Upload Files screen



3. In the Upload Files screen, select **upload RLF**.
>>> The Select RLF Source screen appears ([Figure 2.6](#)).

FIGURE 2.6: Select RLF Source screen



4. In the Select RLF Source screen, the folder and RLF files that are on the memory stick that have not already been uploaded will display as selected. Modify your selection as appropriate and press **next** to upload the file(s).
>>> Your RLF is saved on the local analyzer drive.

Cartridge Definition Files

A Cartridge Definition File, or CDF, defines sample specific data to associate with the data output and defines the parameters for the imaging instrument to use during image collection & processing. Data contained in the CDF includes:

TABLE 2.1: CDF Data

Data Field	Description
Sample Identifier (Sample ID)	The sample ID is a required field. Sample identifiers can be either specific names or can be defaulted to samples 01-12 corresponding to one or more of the 12 flow cells on the sample cartridge. The sample ID is displayed as part of the reporter code count (RCC) file name and as a column heading in the data output file.
Owner Identifier (Owner ID)	The owner ID is an optional field. Examples of use include the name of individual running the experiments or the study's Principle Investigator. This information is output within the corresponding RCC file as general information.
Comments	The comments section is an optional field. Examples of use include experimental condition details regarding the project, or the specific sample. This information is output within the corresponding RCC file as general information.
Reporter Library File (RLF)	This information is required . The RLF file contains the code key information used during image processing to assign target identities to the barcodes. If this file is not designated, the system will not allow data collection. Each RLF is unique to a custom CodeSet so it is critical that the correct RLF be selected during CDF creation.



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

You can create a new CDF file on your desktop or directly on your imaging instrument using the touchscreen. We recommend users create CDFs on their PCs if detailed information for each sample is required. In this case, your memory stick can be used to transfer the CDF to the imaging instrument.

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

- **Scenario 1: Create CDF on your PC** and transfer the file to the analyzer. This is the most practical scenario if detailed and unique information is required for each sample within a cartridge. See [Creating a CDF on Your PC on page 21](#).
- **Scenario 2: Create the CDF on the analyzer and enter data separately** for each tube. This may be required if each sample uses a different CodeSet and required a different RLF, or if the user requires unique information for each sample. This is the most time intensive scenario. See [Creating a CDF on the Digital Analyzer on page 22](#).
- **Scenario 3: Create the CDF on the analyzer and enter data for multiple tubes simultaneously**. See [Creating a CDF on the Digital Analyzer on page 22](#).
- **Scenario 4: Create the CDF on the analyzer and enter data for all tubes and modify information for one tube**. This scenario would be used if most, but not all, data is the same for samples in a single cartridge. See [Creating a CDF on the Digital Analyzer on page 22](#).

To reduce data entry, CDFs can be designed to be assigned multiple sample cartridges. There are many ways to speed data entry but the most rapid method for data entry on the analyzer is to create a template CDF for each CodeSet. To accomplish this, the user would select all tubes, default sample IDs to 01-12, and select an RLF file and press **Done** on the confirmation screen. Save the new CDF with the CodeSet name as the file name. This CDF can be used for any sample cartridge that is used in conjunction with the CodeSet defined in the RLF. However, sample tracking will need to occur in the users lab notebook as no unique identifying information besides the sample cartridge ID will be contained in the data output.

Creating a CDF on Your PC

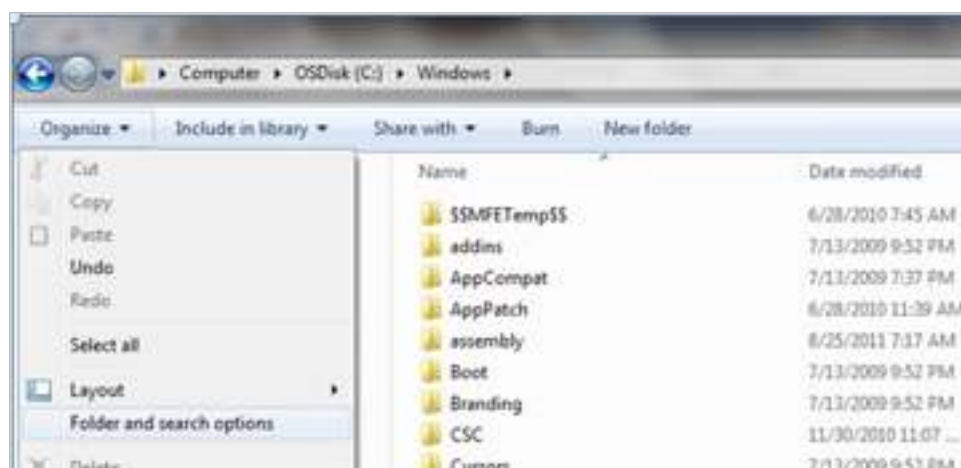
Each memory stick that comes with your new CodeSet has a CDF template loaded onto it in the folder entitled **CDFData**. You can modify this CDF template to contain your sample information and it can be imported and modified in either Microsoft® Excel, or in Notepad for Windows (Textedit for Macintosh®). Steps to do this procedure are provided.

Depending on your system setup, you may need to enable the viewing of file type extensions. If necessary, follow the instructions provided below.

Enable Viewing of File Type Extensions

1. Open an Explorer window and select the **Organize** menu.

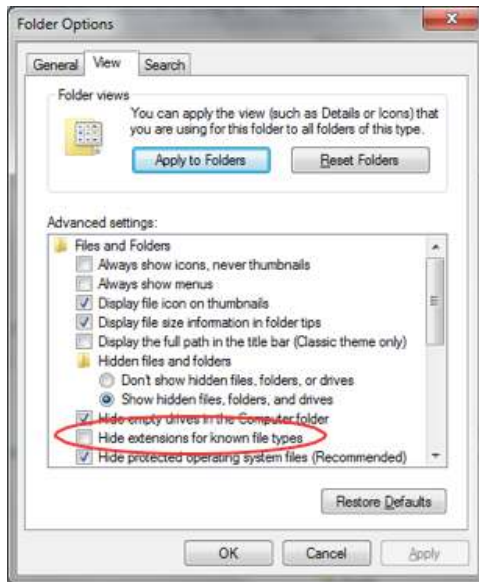
FIGURE 2.7: Organize menu



2. Under the **Organize** menu, select **Folder and Search Options**.
>>> The **Folder and Search Options** window appears.

3. Select the **View** tab, uncheck the option **Hide extensions for known file types**.

FIGURE 2.8: Folder Options window



4. Click the **Apply to Folders** button and click **Apply**.

5. Click **OK**.

To Modify an Existing CDF in Excel

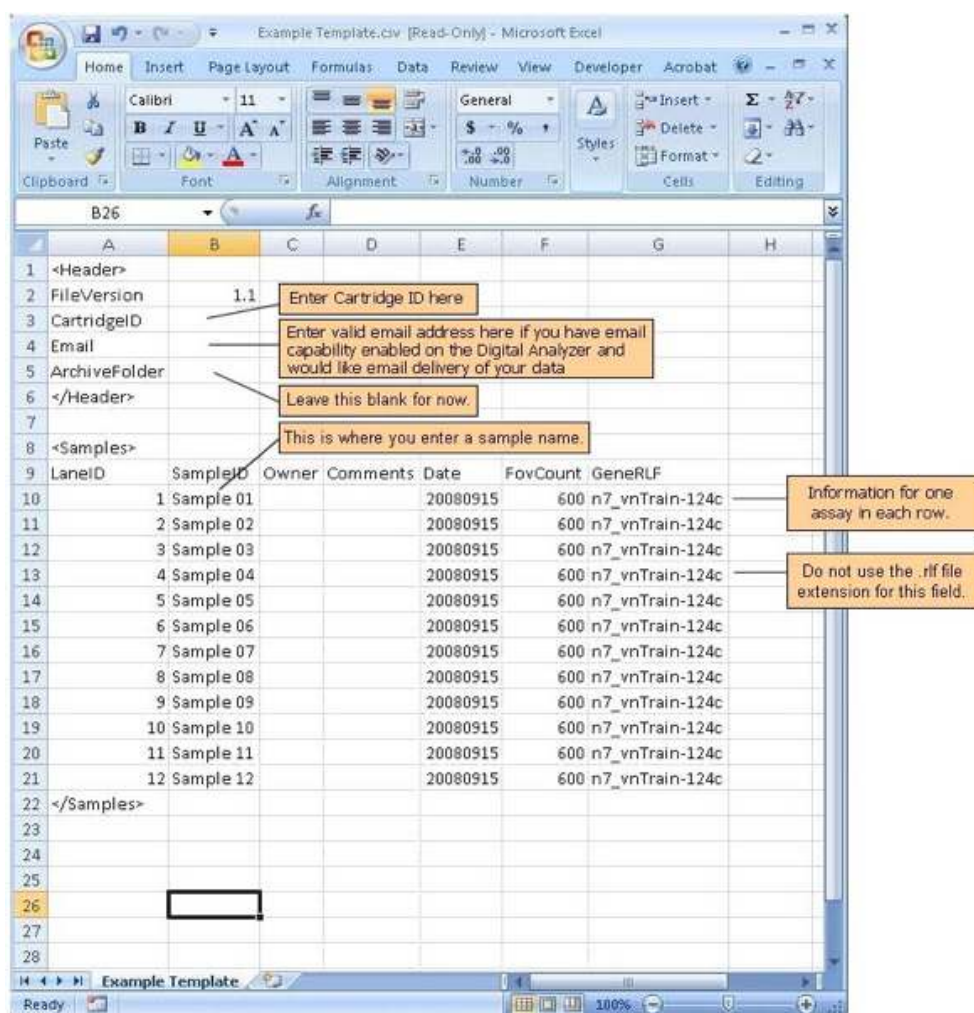
1. Copy the existing CDF template or another CDF file and rename it as desired.

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

- Available characters for renaming a file include:

@ ^ + - # % (~ &)

2. Append ".csv" to the end of the filename.
3. Double-click the file to open it in Excel.
4. Fill in the CDF as shown in **Figure 2.9**.

FIGURE 2.9: Sample CDF in Excel

- **LaneID:** The LaneID column defines which the flow cells in the cartridge will be scanned. If all twelve lanes will be scanned, this should not be changed. If only a subset of lanes will be scanned, the information for the empty lanes can be deleted.
- **Sample ID:** This column is where you may name your samples; technical replicates may be named the same thing.
- **Owner:** This is an optional field which can be named; information is output with the data.
- **Comments:** Enter additional sample or experimental details in the Comments field; information is output with the data.
- **Date:** The Date information is optional and can contain any date in the YYYYMMDD format. The date of a scan is automatically added to the beginning of the RCC file name, so it is not required to add it here.
- **FOVCount:** The number of images to analyze per assay which corresponds to the amount of data to collect. Options are 555, 280, 100, or 25.
- **GeneRLF:** This field defines the reporter library file to associate with the data. It is extremely important that this filename be correct or data will be mis- interpreted. The .rlf file type extension should NOT be used here.

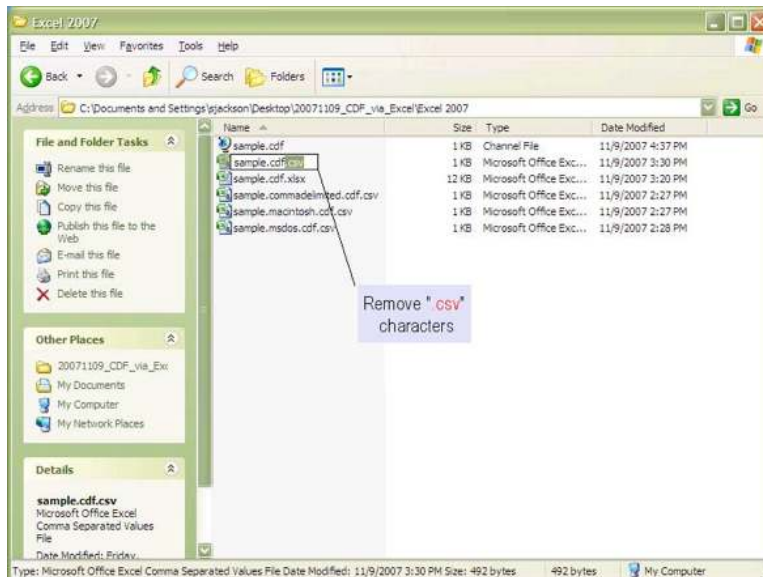
- When finished entering the required information, save as a “.csv” file type (**Figure 2.10**).

FIGURE 2.10: Save As window



- Close the file.
- Remove the extra “.csv” file extension, leaving file name as “*.cdf”, as shown in **Figure 2.11**.

FIGURE 2.11: Removing “.csv” file extension, leaving file name as “*.cdf”



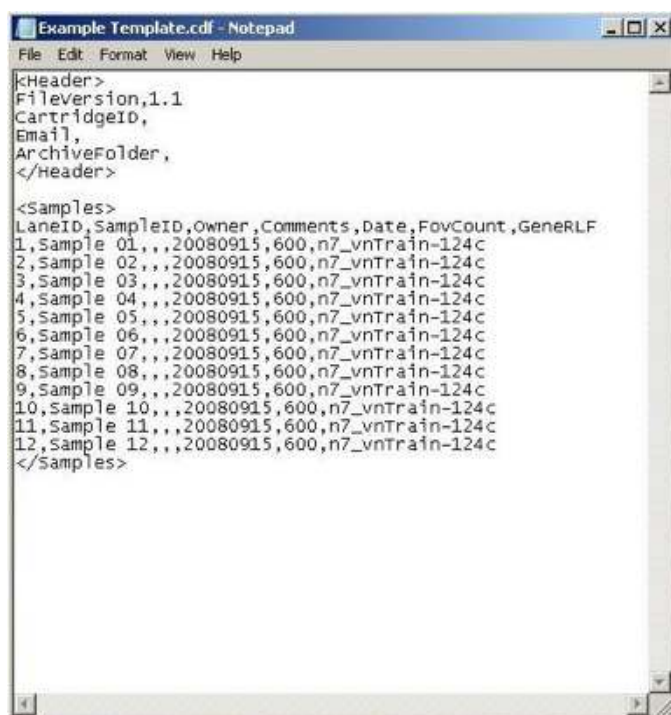
- Transfer the CDF file to the Digital Analyzer using a memory stick. The file is now ready for use by the Digital Analyzer.

NOTE: The CDF file must be placed in the folder on your memory stick entitled **CDFData** in order for the Digital Analyzer to find it.

To Modify a CDF File in Notepad

1. Open the CDF file in Notepad (or Textedit). Note that all the information present in the Excel spreadsheet shown in **Figure 2.12** is visible in Notepad.

FIGURE 2.12: View of CDF file in Notepad



```
<Header>
Fileversion,1.1
CartridgeID,
Email,
ArchiveFolder,
</Header>

<Samples>
LaneID,SampleID,Owner,Comments,Date,FOVCount,GeneRFL
1,Sample 01,,,20080915,600,n7_vnTrain-124c
2,Sample 02,,,20080915,600,n7_vnTrain-124c
3,Sample 03,,,20080915,600,n7_vnTrain-124c
4,Sample 04,,,20080915,600,n7_vnTrain-124c
5,Sample 05,,,20080915,600,n7_vnTrain-124c
6,Sample 06,,,20080915,600,n7_vnTrain-124c
7,Sample 07,,,20080915,600,n7_vnTrain-124c
8,Sample 08,,,20080915,600,n7_vnTrain-124c
9,Sample 09,,,20080915,600,n7_vnTrain-124c
10,Sample 10,,,20080915,600,n7_vnTrain-124c
11,Sample 11,,,20080915,600,n7_vnTrain-124c
12,Sample 12,,,20080915,600,n7_vnTrain-124c
</Samples>
```

2. Enter or change the information appropriate for your experiment:
 - **Under <Header>**
Do not alter any of the existing text. Enter your cartridge ID and 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, GeneRFL). Rather, on a new line, enter your information. The values for these definitions must be separated by commas. Do not use spaces.



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

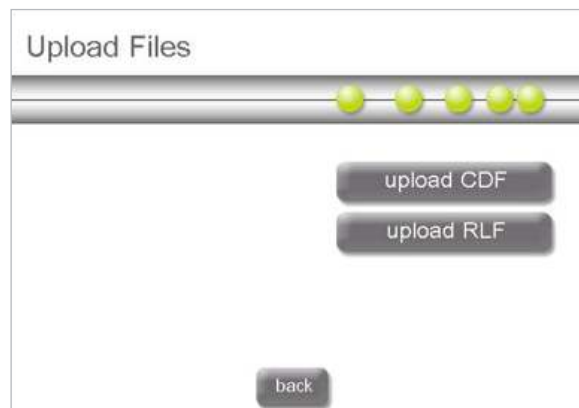
3. Save your file with a .cdf extension.
4. Transfer the CDF file to the Digital Analyzer using a memory stick. The file is now ready for use by the Digital Analyzer.

Uploading Cartridge Definition Files

You can upload CDFs created in your PC from the memory stick to the Digital Analyzer.

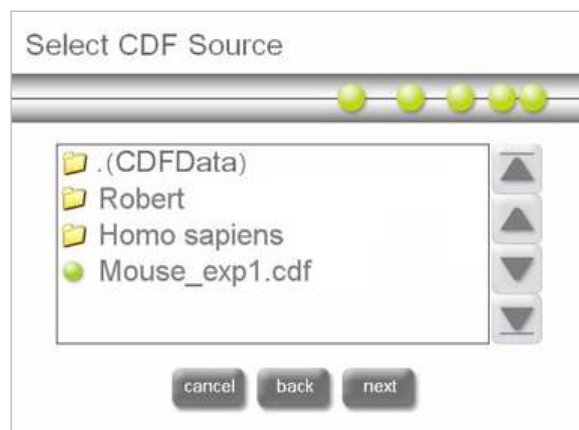
1. Insert the memory stick containing your CDF file saved in the CDFData folder into the USB port on the side of the touchscreen.
2. From the **main menu**, press **Upload Files**.
>>> The Upload Files screen appears (**Figure 2.13**).

FIGURE 2.13: Upload Files screen



3. In the Upload Files screen, select **upload CDF**.
>>> The Select RLF Source screen appears (**Figure 2.14**).

FIGURE 2.14: Select CDF Source screen



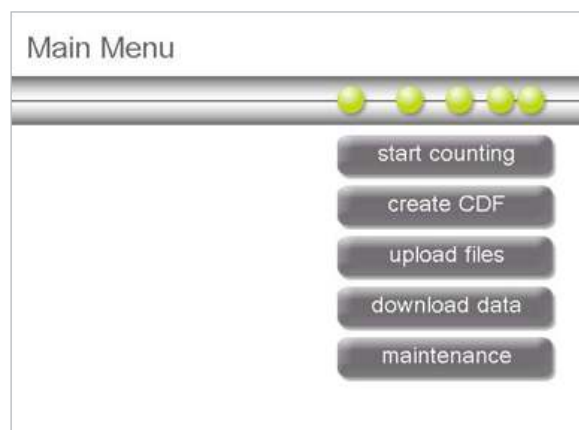
4. In the Select CDF Source screen, browse to the location on your memory stick where the CDF file is stored. Press **next**.
>>> The Select CDF Destination screen appears (**Figure 2.15**).

FIGURE 2.15: Select CDF Destination screen

4. In the Select CDF Destination screen, browse to the folder location on the analyzer where you want to save the CDF. Press **save**.

Creating a CDF on the Digital Analyzer

1. From the Main menu (**Figure 2.16**), press **create CDF**.

FIGURE 2.16: Main menu

>>> The Create Cartridge Definition File screen appears (**Figure 2.17**).

FIGURE 2.17: Main menu

2. In the Create Cartridge Definition File screen, you may begin by clicking in the Cartridge ID field to enter a cartridge ID.

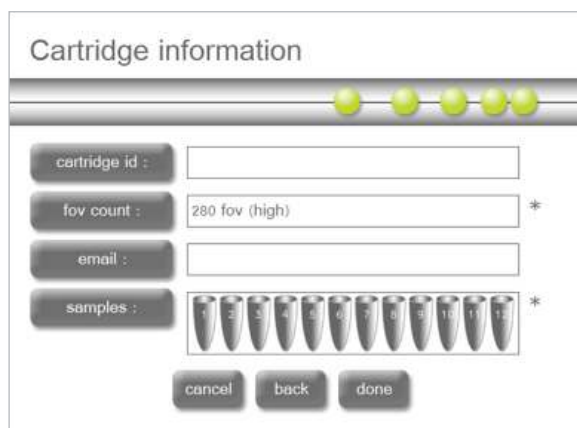
>>> The Enter Cartridge ID screen appears (**Figure 2.18**).

FIGURE 2.18: Enter Cartridge ID screen

3. Enter the Cartridge ID using the touch screen keyboard and click enter when you are done. The cartridge id field is optional in the create CDF workflow. If left empty, you will be prompted to enter the cartridge id after selecting the CDF in the Start Counting workflow.

>>> This will bring you back to the Cartridge Information screen

FIGURE 2.19: Cartridge information screen

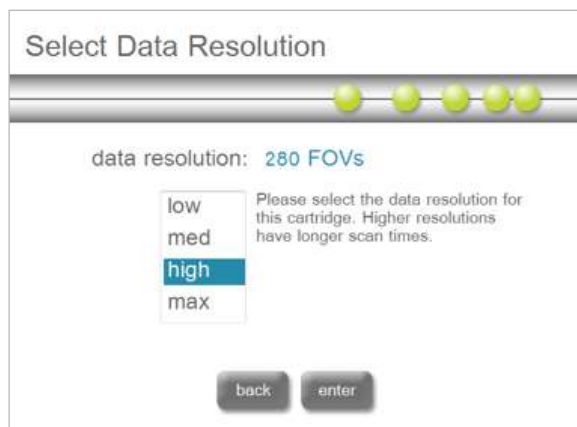


The 'Cartridge information' screen features a title bar with four green indicator lights. Below the title, there are four input fields: 'cartridge id' (empty), 'fov count' (set to '280 fov (high)' with an asterisk), 'email' (empty), and 'samples' (a row of 12 sample slots, each with a number 1-12, followed by an asterisk). At the bottom are three buttons: 'cancel', 'back', and 'done'.

4. Click on the FOV count field.

>>> The Select Data Resolution Entry screen appears (**Figure 2.20**).

FIGURE 2.20: Select Data Resolution Entry screen



The 'Select Data Resolution' screen has a title bar with four green indicator lights. It displays 'data resolution: 280 FOVs'. Below this is a list box with options: 'low', 'med', 'high' (highlighted in blue), and 'max'. To the right of the list box is a note: 'Please select the data resolution for this cartridge. Higher resolutions have longer scan times.' At the bottom are two buttons: 'back' and 'enter'.

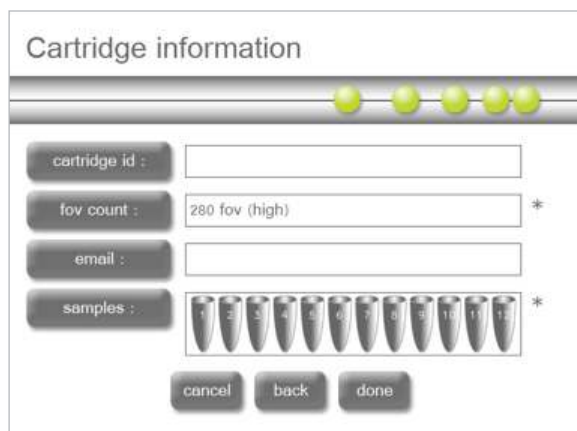
In the Select Data Resolution screen, select the desired data resolution. The number of images collected corresponds to the sensitivity and dynamic range that can be achieved. If you prefer to have higher throughput on the analyzer, you may opt to collect fewer images if sensitivity is not as important to your application. For example, if you wish to rescan a cartridge to check for data quality a low resolution scan takes approximately one minute per flow cell vs. 20 minutes per flow cell at the maximum resolution.

- **low** resolution takes 25 images per sample
- **medium** resolution takes 100 images per sample
- **high** resolution takes 280 images per sample
- **max** resolution takes 555 images per sample (**Note:** Specifications for this system re based on the “high” data resolution setting. Therefore, the “max” resolution setting does not guarantee that the throughput specification will be met.)

5. Press **enter**.

>>> This will bring you back to the Cartridge Information screen

FIGURE 2.21: Cartridge information screen




The 'Cartridge information' screen features a title bar with five green indicator lights. Below the title, there are four input fields: 'cartridge id' (empty), 'fov count' (set to '280 fov (high)' with an asterisk), 'email' (empty), and 'samples' (a row of 12 numbered slots, with an asterisk). At the bottom are three buttons: 'cancel', 'back', and 'done'.

5. Click on the **email** field.

>>> The Select Email Address screen appears

FIGURE 2.22: Select Email Address Entry screen



The 'Select email address' screen has a title bar with five green indicator lights. It displays a list of email addresses: 'jowen@nanosttring.com' and 'lirvin@nanosttring.com'. To the right of the list are four navigation buttons: up, down, and two additional buttons. At the bottom are four buttons: 'back', 'none', 'enter', and 'add email'.

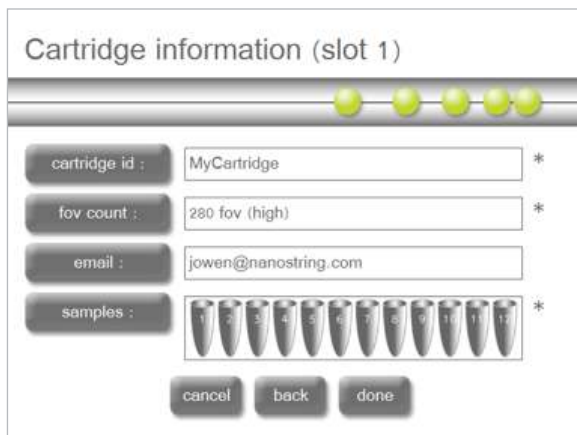
Select an email address where the data from a cartridge will be sent. The data will be stored in the 'older data' section of the Digital Analyzer after emailing and can be retrieved using a memory stick or network connection if so configured. Email must be configured on the system before using this option for data delivery (see [Email Address on page 42](#) for additional information on this and entering new email addresses).

NOTE: Email must be configured first in order to allow this function to work. Setting an email from this screen will not automatically email results unless email is configured. To configure email see **Email Address section on page 42**.

7. Click **enter**.

>>> This will bring you back to the Cartridge Information screen

FIGURE 2.23: Cartridge information screen

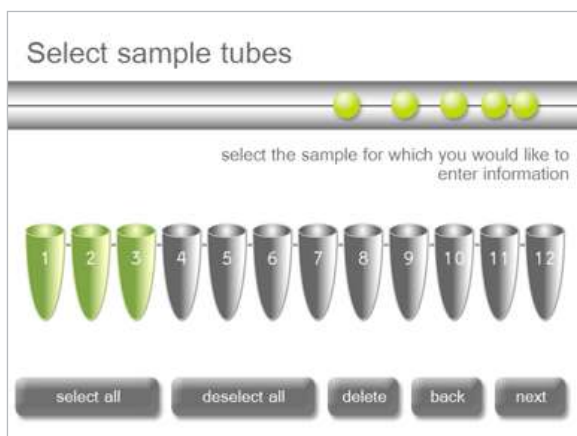


The screenshot shows the 'Cartridge information (slot 1)' screen. At the top, there is a title bar with the text 'Cartridge information (slot 1)' and a progress indicator consisting of five yellow circles. Below the title bar, there are four input fields, each with a label and a value, and an asterisk indicating a required field. The fields are: 'cartridge id : MyCartridge', 'fov count : 280 fov (high)', 'email : jowen@nanostring.com', and 'samples :'. The 'samples' field is a dropdown menu showing 12 sample tubes. At the bottom of the screen, there are three buttons: 'cancel', 'back', and 'done'.

8. Click on the **samples** field.

>>> The select sample tubes screen appears

FIGURE 2.24: Select sample tubes screen



The screenshot shows the 'Select sample tubes' screen. At the top, there is a title bar with the text 'Select sample tubes' and a progress indicator consisting of five yellow circles. Below the title bar, there is a text prompt: 'select the sample for which you would like to enter information'. Below the prompt, there are 12 sample tubes, numbered 1 through 12. Tubes 1, 2, and 3 are highlighted in green, indicating they are selected. At the bottom of the screen, there are five buttons: 'select all', 'deselect all', 'delete', 'back', and 'next'.

9. Select the tubes for which you would like to enter sample information for by touching the appropriate tube on the screen. To select all tubes and enter the same information for all, click the **select all** button. Selected samples are highlighted in green. Click next to proceed.

>>> The Sample tubes screen appears

FIGURE 2.25: Sample tubes screen

10. In **Figure 2.25**, the available sample definition fields are displayed. Select each button or box to open a keyboard screen to enter data. Enter the following information for each field using the keyboard and click enter to return to the sample tubes screen:

- a. In the sample ID data field enter a unique sample ID (if a single tube is selected).
- b. In the owner ID data field enter the owner ID.
- c. In the comments data field enter useful comments.
- d. Click on the library RLF data field.

>>> The select Reporter Library File screen appears

FIGURE 2.26: Select Reporter Library file screen

- e. In the Select Reporter Library File screen (**Figure 2.26**), select the RLF file to use with this sample and press enter. The RLF contains the information necessary to decode the digital color coded reporter signals into their target identities.



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

After all information for selected fields are completed press done.

>>> This sample information is outputted with the RCC data and can be used for parsing or annotating data.

>>> The Cartridge Information screen appears with the defined samples displayed in blue. (**Figure 2.27**).

FIGURE 2.27: The Cartridge information screen

The Cartridge information screen displays a title bar with five green spheres. Below the title bar, there are four input fields with labels: 'cartridge id :', 'fov count :', 'email :', and 'samples :'. The 'cartridge id' field contains 'li0001CART'. The 'fov count' field contains '280 fov (high)' and has an asterisk. The 'email' field contains 'jowen@nanosttring.com'. The 'samples' field contains a row of 12 sample icons, each labeled with a number from 1 to 12, and has an asterisk. At the bottom, there are three buttons: 'cancel', 'back', and 'done'.

11. For **Figure 2.27**, 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 9-10, until all desired tubes in cartridge have sample information defined. When all samples for the cartridge are defined, press **done**.

>>> The Select CDF Destination screen appears (**Figure 2.28**).

FIGURE 2.28: The Select CDF Destination screen

The Select CDF Destination screen displays a title bar with five green spheres. Below the title bar, there is a list box containing two items: 'LI Folder' and 'LI CDF 100 .cdf'. To the right of the list box are four navigation buttons: up, down, left, and right. At the bottom, there are four buttons: 'cancel', 'back', 'next', and 'new folder'.

12. In the Select CDF Destination screen, select an existing folder or create a new folder in which to save the CDF.

- You can navigate the folder structure by touching folders twice, similar to double-clicking with a mouse.

13. Press **next**.

>>> The Enter New CDF Name screen appears (**Figure 2.29**).

FIGURE 2.29: The enter new cdf name screen

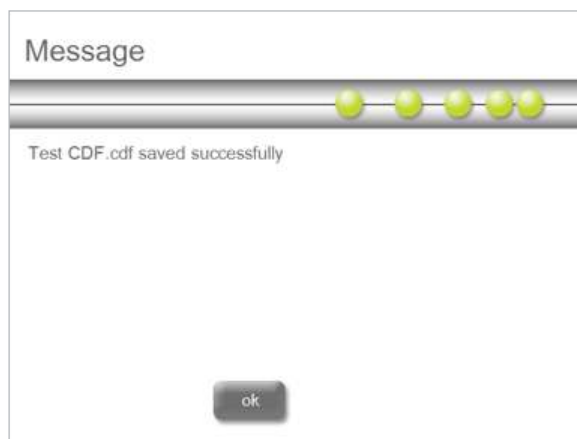


14. Enter the CDF Name using the keyboard and press **enter**.

>>> If the CDF name already exists, an error will display and you will be asked if you want to overwrite the existing file. If you select **no**, you can then change the name of the CDF file.

>>> A confirmation message appears (**Figure 2.30**).

FIGURE 2.30: The enter new cdf screen



15. From the Main menu, you can now select **start counting**, using the CDF that was just created and saved (See **Starting a Run on page 32**).

Starting a Run

This section guides you through starting a run on the Digital Analyzer.

1. From the Main menu (Figure 2.31) select **start counting**.

>>> The Select Stage Positions screen appears (Figure 2.32).

FIGURE 2.31: Main menu

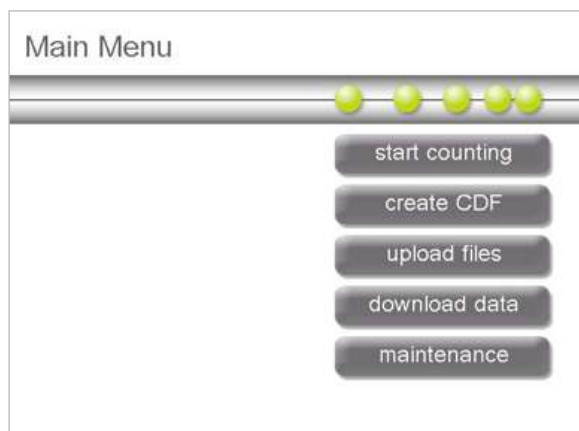
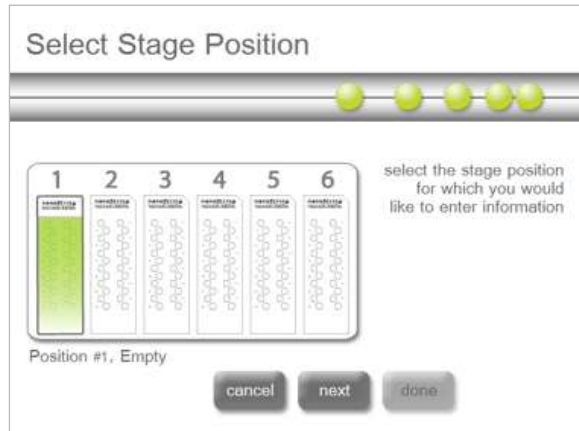


FIGURE 2.32: The Select Stage Position screen

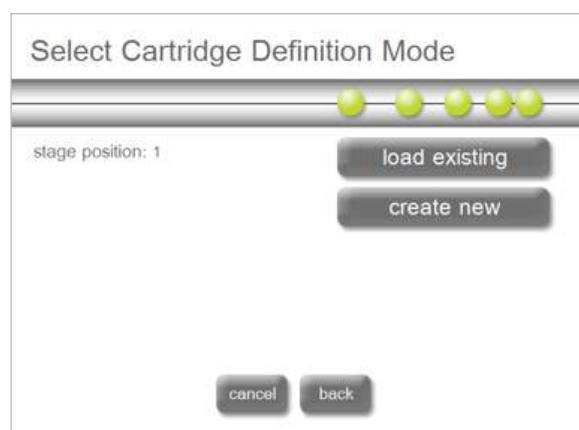


2. In the Select Stage Position screen, select the cartridge position for which you would like to enter cartridge information by touching the cartridge position on the screen.

>>> The selected cartridge appears in green. If you selected the wrong cartridge, simply touch the correct position and the active cartridge will display in the new position.

3. Press **next**.

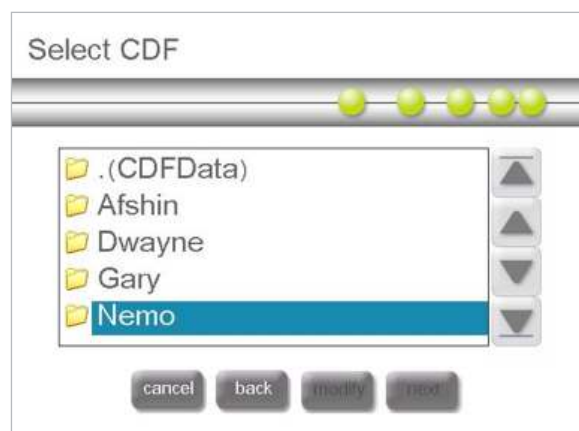
>>> The Select Cartridge Definition Mode screen appears (Figure 2.33).

FIGURE 2.33: The Select Cartridge Definition Mode screen

4. In the Select Cartridge Definition Mode screen, you can select an existing CDF or create a new CDF. If you have created a CDF and uploaded/saved it to the system, press **load existing**.

A CDF is a file that defines the parameters to use during image collection and processing and defines the sample-specific data to associate with the data output. The following example demonstrates selecting an existing CDF.

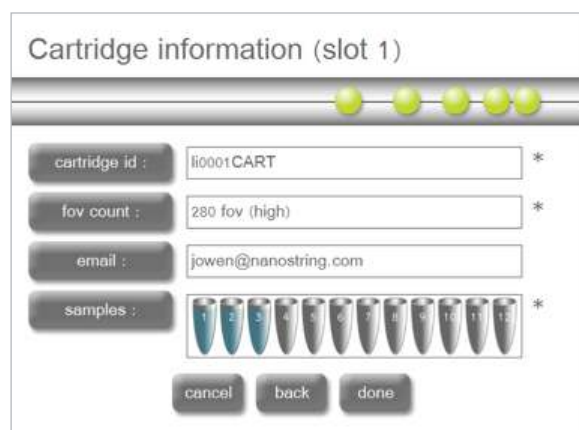
>>> The Select CDF screen appears (**Figure 2.34**).

FIGURE 2.34: The Select CDF screen

5. In the Select CDF screen, select the CDF you want to use and press **next**.

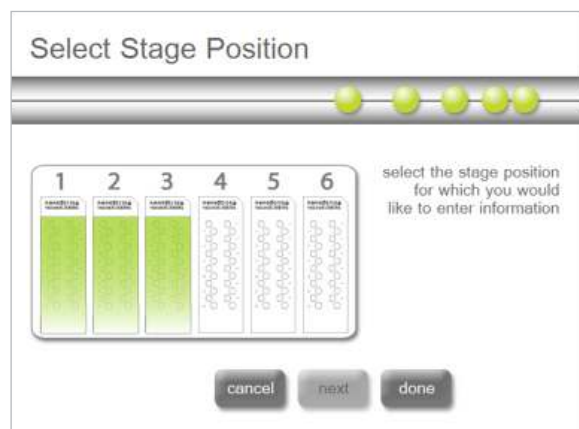
>>> The Cartridge Information screen appears (**Figure 2.35**).

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

FIGURE 2.35: The Cartridge Information screen

The screenshot shows the 'Cartridge information (slot 1)' screen. At the top, there is a title bar with the text 'Cartridge information (slot 1)' and a progress indicator consisting of five green circles. Below the title bar, there are four input fields, each with a label and an asterisk: 'cartridge id :', 'fov count :', 'email :', and 'samples :'. The 'cartridge id' field contains 'li0001CART'. The 'fov count' field contains '280 fov (high)'. The 'email' field contains 'jowen@nanostring.com'. The 'samples' field contains a row of 12 small vial icons, numbered 1 through 12. Below the input fields are three buttons: 'cancel', 'back', and 'done'.

6. To change any of the information specified for the cartridge, press any of the appropriate fields on the screen and follow the instructions as specified in Steps 2-10 of Section – Creating a CDF on the Digital Analyzer.
7. Press **done**.
 >>> The Select Stage Position screen appears (Figure 2.36).
8. Continue to repeat Steps 2-7 above until all cartridges for the run are defined.

FIGURE 2.36: The Select Stage screen

The screenshot shows the 'Select Stage Position' screen. At the top, there is a title bar with the text 'Select Stage Position' and a progress indicator consisting of five green circles. Below the title bar, there are six columns, each labeled with a number from 1 to 6. Each column contains a series of small vial icons. To the right of the columns, there is a text prompt: 'select the stage position for which you would like to enter information'. Below the columns are three buttons: 'cancel', 'next', and 'done'.

9. Press **done**.

10. Place cartridges in the orientation shown in **Figure 2.37** and **Figure 2.38** into the stage position corresponding to your data entry. Be sure that the cartridge is seated flat in the slot and close the magnetic clips gently.

FIGURE 2.37: Proper orientation of cartridges when placing them into the Digital Analyzer



FIGURE 2.38: Close the magnetic clips gently

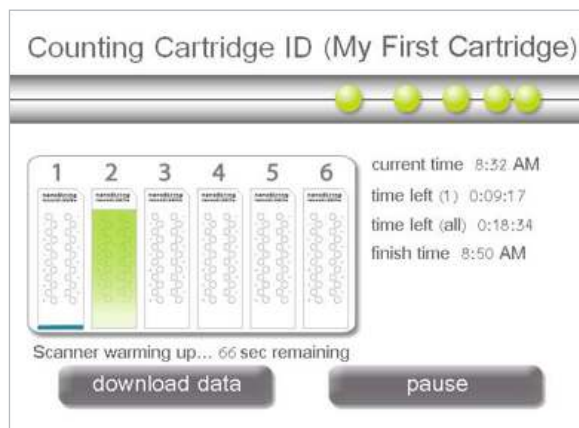


11. Shut the instrument door. In the Initiate Imaging screen, press **start**.

>>> The imaging begins. Once imaging has begun, the door remains locked until the system is paused or the run is complete.

>>> The Counting Cartridge ID screen appears (**Figure 2.39**).

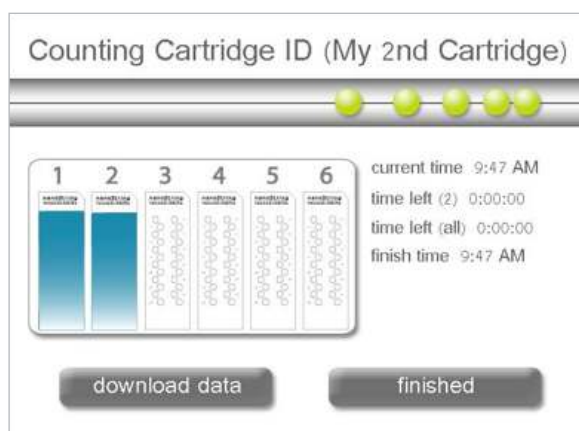
FIGURE 2.39: The Counting Cartridge ID screen - with run in progress information



Once imaging begins, the Counting Cartridge ID screen displays the following information:

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

FIGURE 2.40: The Counting Cartridge ID screen - with completed run information



The screen image in [Figure 2.40](#) displays information for a completed run.

COMMAND/BUTTON Options

- **download data:** Press 'download data' to access data that has already been collected while the Digital Analyzer is still running. For instructions on downloading files, see [Retrieving Reporter Code Count Files on page 44](#).
- **pause:** Use this button 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 you to the main menu.

Pausing a Run

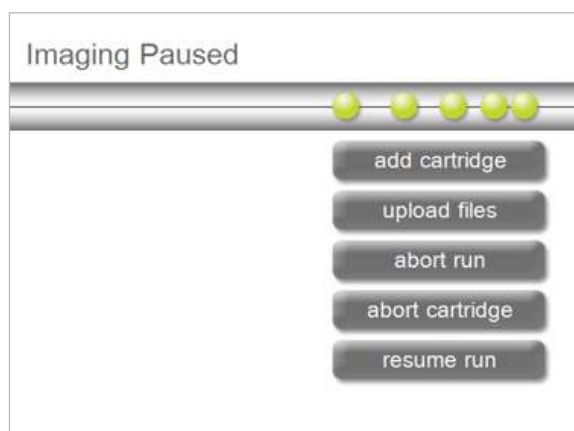
From the Counting Cartridge screen you can pause the run to add additional cartridges, and you can abort the run.

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

To Add a New Cartridge

1. In the Counting Cartridge ID screen ([Figure 2.39](#)) press **pause**.
>>> The Imaging Paused screen appears after a period of time ([Figure 2.41](#)).

FIGURE 2.41: Imaging Paused screen



2. Press **add cartridge**.
3. After completing the add a cartridge workflow (identical to defining a new cartridge as described above), press **resume run** when you are ready to continue processing.

To Abort a Run

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

1. In the Counting Cartridge ID screen (Figure 2.39) press **pause**.

>>> The Paused screen appears (Figure 2.42).

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

NOTE: If you choose to abort a run but want to re-image cartridges, simply set up a new run with the same cartridges following the instructions outlined in **Starting a Run on page 26**.

>>> The Abort Confirmation screen appears (Figure 2.43).

FIGURE 2.43: The Abort Confirmation screen



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

To Abort a Cartridge

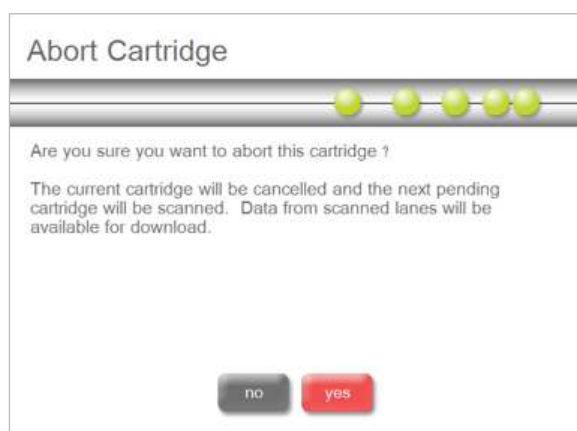
When you abort a cartridge, data on cartridges and flow cells that have completed imaging is saved and available for download.

1. In the Counting Cartridge ID screen (**Figure 2.39**) press **pause**.
 >>> The Paused screen appears (**Figure 2.42**).
2. To abort the cartridge, but not the entire run, press **abort cartridge**.

NOTE: If you choose to abort a run but want to re-image cartridges, simply set up a new run with the same cartridges following the instructions outlined in **Starting a Run on page 26**.

>>> The Abort Confirmation screen appears (**Figure 2.44**).

FIGURE 2.44: The Abort Confirmation screen



3. Press **yes** to abort the cartridge.

To Upload Files

The Digital Analyzer can be paused in order to upload a new CDF or RLF file.

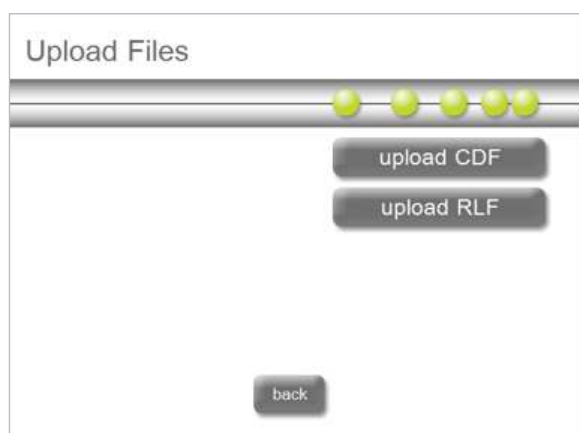
1. In the Counting Cartridge ID screen (**Figure 2.39**) press **pause**.

>>> The Paused screen appears (**Figure 2.42**).

2. To upload new CDF or RLF files, press **upload files**.

>>> The Upload files screen appears (**Figure 2.45**).

FIGURE 2.45: The Upload Files screen



3. Follow instructions to Upload Files starting on page 26.

Completing a Run

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

Data Output

A Reporter Code Count (RCC) file is created for each flow cell in the cartridge. RCC files are automatically named by the Digital Analyzer by 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 can be accessed by following the instructions outlined in the download data section.

Retrieving Reporter Code Count Files

Reporter Code Count files may be retrieved in one of four ways. Directions for each method are provided.

- Transfer via memory stick
- Email delivery of data
- FTP transfer from the Digital Analyzer to the user's PC
- SSH transfer from the Digital Analyzer to the user's PC

Transfer RCC Files via Memory Stick

1. From the Main menu (**Figure 2.46**), press **download data**.

>>> The Select Data to Download screen appears (**Figure 2.47**).

FIGURE 2.46: The Main menu

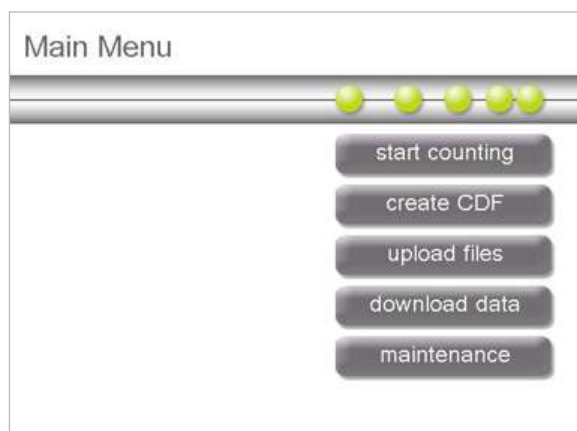
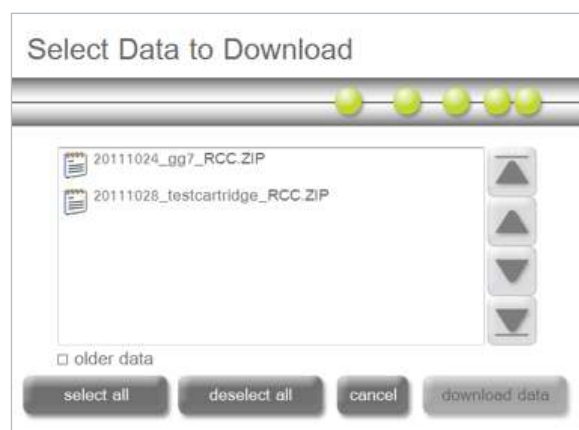


FIGURE 2.47: The Select Data to Download screen

2. In the Select Data to Download screen, you may select data for download to your memory stick RCCData folder. All data that has been processed and is available is displayed in this screen until it has been downloaded once, after which it is moved to the 'older data' page.

Selecting Files for Download

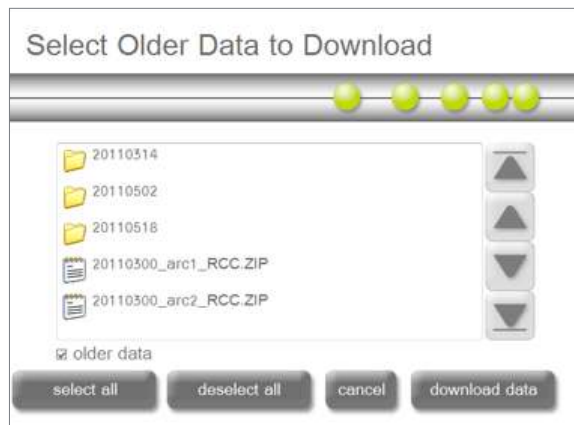
- a. To select a file, touch the file name on the screen. You may select multiple files.
>>> Your selections turn blue.
- b. To de-select a file, touch it on the screen again.
>>> The deselected files turn back to white.
- a. After you have selected the file(s) you want to download, press **download data**.

Selecting “Older Data” Files

If you have trouble finding your data in the top level window, they may be located in the Older Data section.

NOTE: Do not select the root folder for data download as this folder may contain a large quantity of data (RecycleBin).

- a. In the Select RCC Source screen, select the older data option box (located below the file selection area).
>>> The Select Older Data to Download screen appears displaying all “older data files” available for download (**Figure 2.48**).

FIGURE 2.48: The Select Older Data to Download screen

- b. To select a file, touch the file name on the screen. You may select multiple files.

>>> Your selections turn blue.

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

>>> The deselected files turn back to white.

- d. After you have selected the file(s) you want to download, press **download data**.

Older data is saved by day in dated folders and is identified by a date format (YYYYMMDD). You may select to download all of the data from that day or open the dated folder by double touching and selecting only the cartridge of interest. To go back to the top directory level, press **(RecycleBin)**. Files are not removed from the recycle bin unless actively cleaned out.

Email Delivery of RCC Data Files

If you have enabled Digital Analyzer networking and email via the maintenance screens AND have entered a valid email address either into a CDF template or during setup of a run, RCC data from that cartridge will be emailed to you. A copy of the data will be saved in the “older data” folder on the digital analyzer once the data has been emailed. Please be aware that not all institutions allow .zip files to be emailed. If you are having trouble receiving your data, please contact your IT department and verify that .zip files are permitted.

SSH Transfer of RCC Data Files to the User’s PC

If you prefer to use SSH rather than FTP to transfer data files, this can be configured on the Digital Analyzer by NanoString personnel. Contact NanoString at support@nanosttring.com to have SSH configured and enabled on the Digital Analyzer.

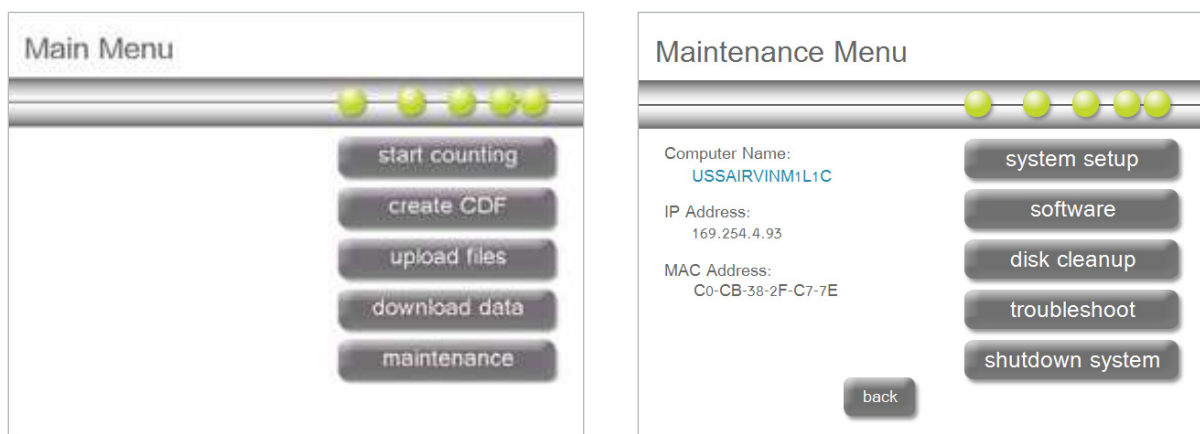
FTP Transfer of RCC Files from the Digital Analyzer to the User’s PC

You will need to know your IP Address and FTP Password prior to transferring data from the Digital Analyzer to your PC.

IP Address Location

Go to the maintenance menu on the Digital Analyzer and write down the IP address. To access the Maintenance menu press maintenance on the Main menu (**Figure 2.49**).

FIGURE 2.49: The Main menu and Maintenance menu



FTP Password

For information on configuring your FTP password, please refer to **FTP Setup on page 48**.

FTP Data Transfer

These instructions cover a single method for retrieving data from the Digital Analyzer. However there are many FTP applications available, any of which should work for transferring data from the Digital Analyzer to your PC.

1. On the user's PC, open a browser window and type in:
FTP://(your IP address here) as shown in **Figure 2.50**.

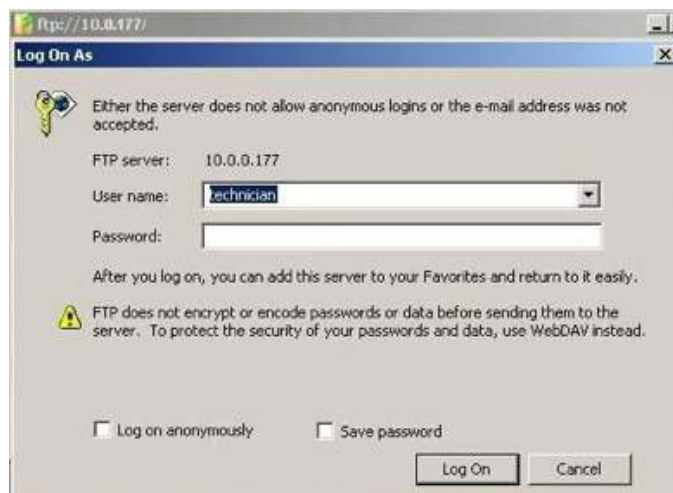
FIGURE 2.50: Entering IP address in browser



2. Press **Enter**.

>>> The FTP Log On window appears (**Figure 2.51**).

FIGURE 2.51: FTP Logon window



3. Enter 'technician' into the FTP **User name** and enter your password as defined in the FTP setup utility through the maintenance menu, then click the **Log on** button.

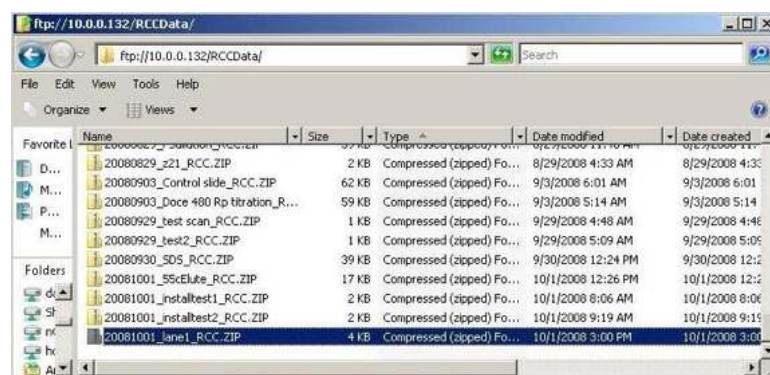
>>> The FTP Access window appears (**Figure 2.52**).

FIGURE 2.52: FTP Access window



4. Open the RCCData folder.

>>> The RCCData folder opens and displays the available data (**Figure 2.53**).

FIGURE 2.53: RCCData folder window

5. Drag and drop data from the RCCData folder onto your desktop.

NOTE: RCC files can be downloaded using FTP, but files cannot be uploaded to the Digital Analyzer using FTP.

3

Maintenance and System Setup

This chapter contains specific information regarding the maintenance and system setup procedures for the nCounter Digital Analyzer.

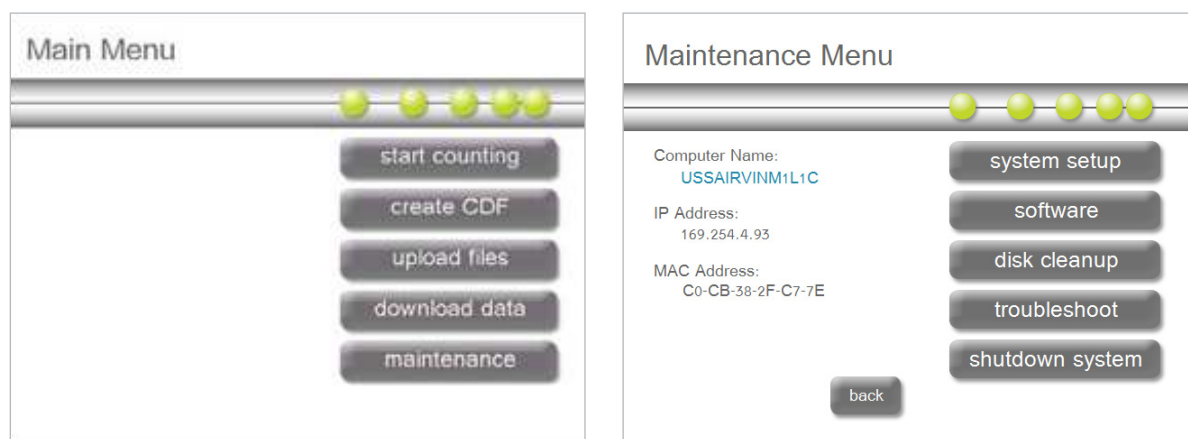
Maintenance Menu Tasks

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

- **system setup:** set system clock, email settings, and FTP password
- **software:** update Digital Analyzer's system software when a new release occurs, view the current software version information, and restart the system in troubleshooting situations.
- **disk cleanup:** delete older CDF, RLF and RCC files
- **troubleshoot:** download log files for remote analysis at NanoString
- **shutdown system:** System shutdown should be used when the system needs to be shutdown. Use this button before reaching around the back and flipping the switch off.

Additionally, you can view the Computer Name, IP Address, and MAC Address of the Digital Analyzer in the maintenance menu. To access the Maintenance menu press maintenance on the Main menu (**Figure 3.1**).

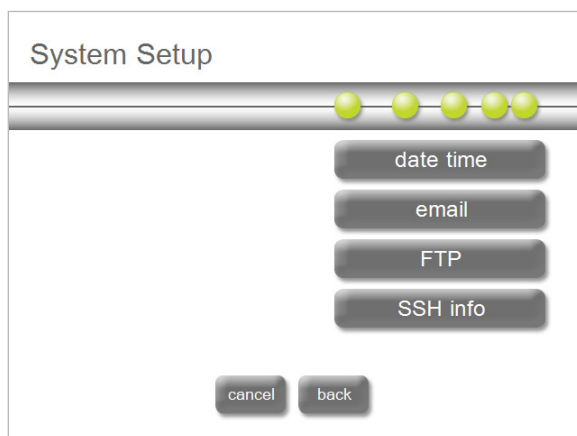
FIGURE 3.1: The Main menu and Maintenance menu



System Setup

The System Setup menu in the Maintenance section allows you to set the Digital Analyzer's date and time, configure email settings, and set the FTP server password.

FIGURE 3.2: 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 3.3: Set Date and Time screen



Email Address

The system can send data to your email account if this option is configured. In the setup screen you are 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 zipped into a folder and emailed to the account listed for each cartridge.

FIGURE 3.4: Email Setup screens

The figure displays two side-by-side screenshots of the 'Email configuration' setup screens. Both screens feature a title bar with the text 'Email configuration' and a progress indicator with five green dots. The left screen contains the following fields: 'smtp server' (with an asterisk), 'smtp port' (with the value '25' and an asterisk), 'from address' (with an asterisk), and 'from security' (with the value 'disabled'). The right screen contains the following fields: 'from address' (with an asterisk), 'from security' (with the value 'disabled'), 'from password', and 'test address'. Both screens have a vertical stack of four arrow buttons (up, down, left, right) on the right side. At the bottom of each screen are four buttons: 'cancel', 'test', 'save', and 'email accounts'.

NOTE: Please contact your IT personnel for assistance in setting up email as an option on the Digital Analyzer.

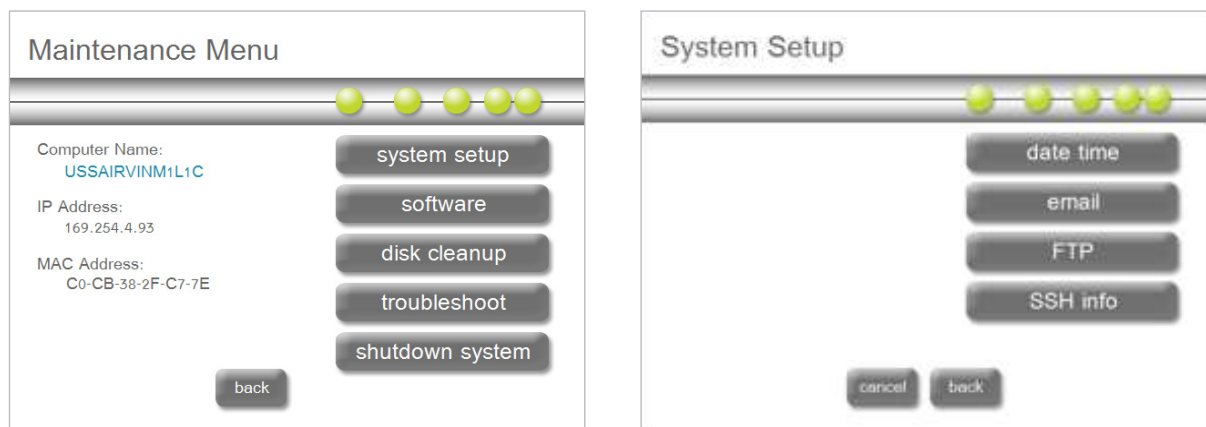
- **SMTP server:** Simple Mail Transport Protocol (SMTP) is the network protocol used to send email across the Internet. The SMTP server setting must be configured correctly or outgoing mail will not be sent. Enter the name of your SMTP server for outgoing mail.
- **SMTP port:** Enter the name of the port used by your SMTP server.
- **from address:** Enter a valid email address. This address will be seen in the "From" line of any outgoing email correspondences with NanoString.
- **from security:** Security is disabled by default. To enable security on the Digital Analyzer and enter a 'from' password, press from security.
- **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: It is recommended that you test the email settings prior to setting up a run by selecting the 'test' button and checking the inbox of the test email address.

FTP Setup

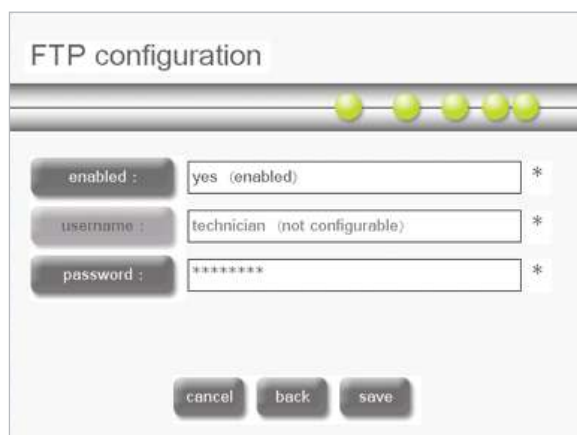
1. To configure FTP access to the Digital Analyzer, select FTP on the system setup menu. (Figure 3.6).

FIGURE 3.6: The Maintenance menu and System Setup menu



>>> The FTP Settings screen appears (Figure 3.7).

FIGURE 3.7: FTP configuration



The default **FTP Username** is set to “technician”. The Username cannot be changed.

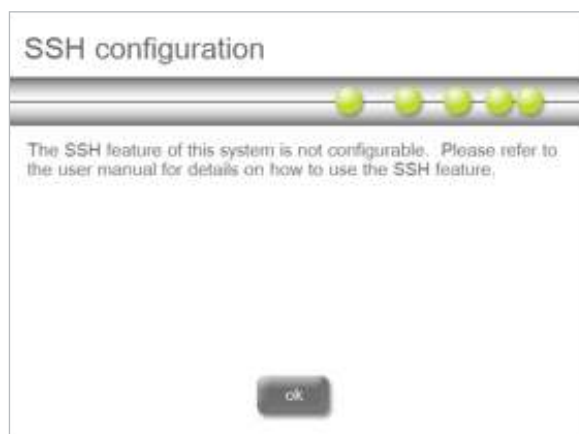
2. To set or change the password at any time, start typing another password and it will overwrite the existing one. Press **save**.
3. If you do not wish to enable FTP access to the Digital Analyzer, press the **enabled** box so that it displays “no (disabled)”, and no FTP access will be allowed.

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

SSH Setup

1. If you prefer to use SSH rather than FTP to transfer data files, this can be configured on the Digital Analyzer by NanoString personnel. Contact NanoString at support@nanosttring.com to have SSH configured and enabled on the Digital Analyzer.

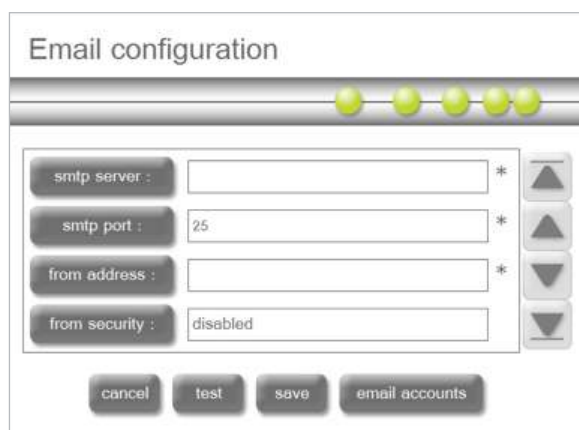
FIGURE 3.8: The SSH Configuration screen



Email Accounts

Email addresses can be added, deleted, managed and saved on the Digital Analyzer by pressing **email accounts** from the email configuration screen. Email accounts can also be added from the Cartridge Information screen when creating a CDF or can be added from the Email Configuration screen by pressing **email accounts**.

FIGURE 3.9: Email Configuration screen



>>> The Manage email addresses screen appears (**Figure 3.10**).

FIGURE 3.10: The Manage Email Addresses screen



1. To add an email address, press **add**.

>>> The Enter email address screen appears (**Figure 3.11**).

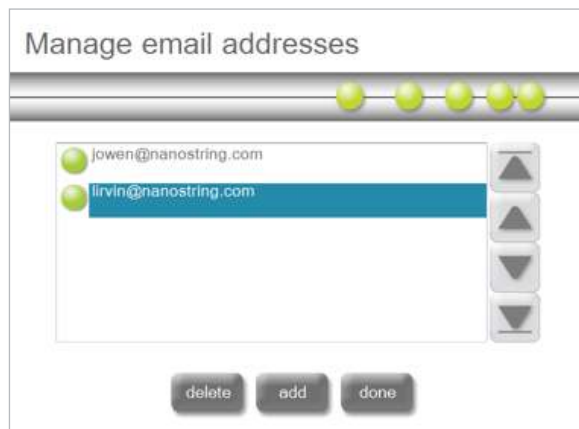
FIGURE 3.11: The Enter Email Addresses Keyboard screen



2. Enter a valid email address and press enter. The email address gets saved.

>>> The Manage email addresses screen appears (**Figure 3.12**).

FIGURE 3.12: The Manage Email Addresses Screen

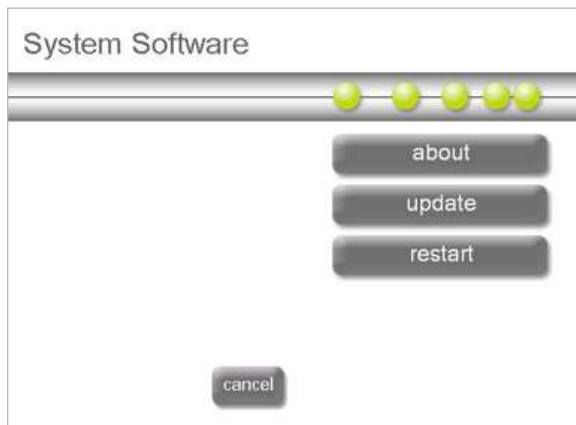


3. To delete an email address press the email address on the touchpad and press **delete**.
4. When you are finished, press **done**.

Software

Use the **software** section under 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 3.13: System Software screen



Should you need assistance with your Digital Analyzer, please identify system software version prior to calling NanoString. This information can be found in the about section on the System Software menu.

FIGURE 3.14: About screen



Update

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

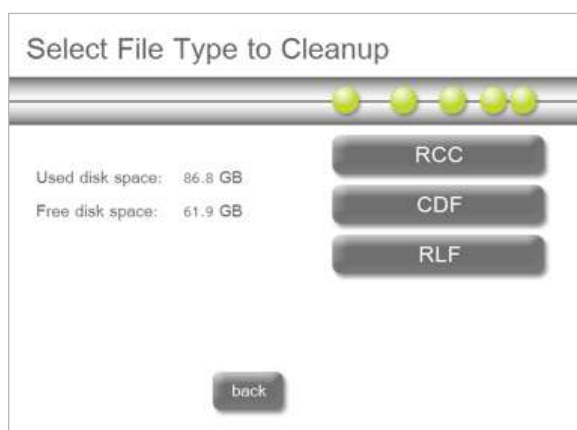
Restart

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

Disk Cleanup

Use the Disk Cleanup option to check the current disk space usage (used and free), to delete RCC files, to delete CDF files, and to delete RLF files.

FIGURE 3.15: Disk Cleanup screen



Cleanup RCC Files

1. To cleanup RCC files, press **RCC** in the Disk Cleanup screen.
>>> The Select Files to Delete screen appears ([Figure 3.16](#)).

FIGURE 3.16: Select Files to Delete screen



2. Select the files you want to delete and press **delete**.
3. At the prompt, confirm the files to delete by pressing **yes**.

Cleanup CDF Files

1. To cleanup CDF files, press **CDF** in the Disk Cleanup screen.
>>> The Select Files to Delete screen appears ([Figure 3.17](#)).

FIGURE 3.17: Select Files to Delete screen



2. Select the files you want to delete and press **delete**.
3. At the prompt, confirm the files to delete by pressing **yes**.

Cleanup RLF Files

1. To cleanup RLF files, press **RLF** in the Disk Cleanup screen.
>>> The Select Files to Delete screen appears (**Figure 3.18**).

FIGURE 3.18: Select Files to Delete screen



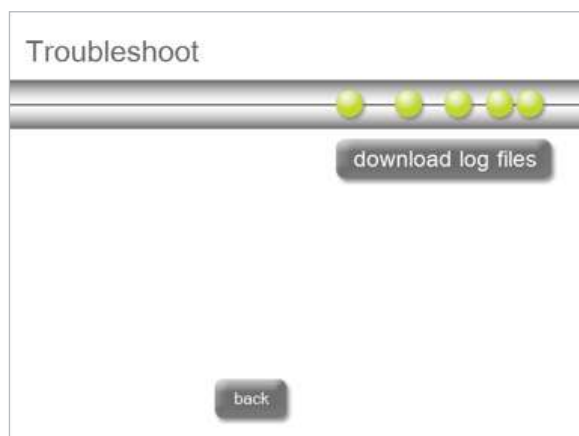
2. Select the files you want to delete and press **delete**.
3. At the prompt, confirm the files to delete by pressing **yes**.

Troubleshooting

Use the Troubleshooting screen to download log files to send to NanoString when there is a problem with the results you are getting that cannot be identified.

1. Press **Troubleshoot** from the Maintenance screen.
>>> The **Troubleshoot** screen appears (**Figure 3.19**).

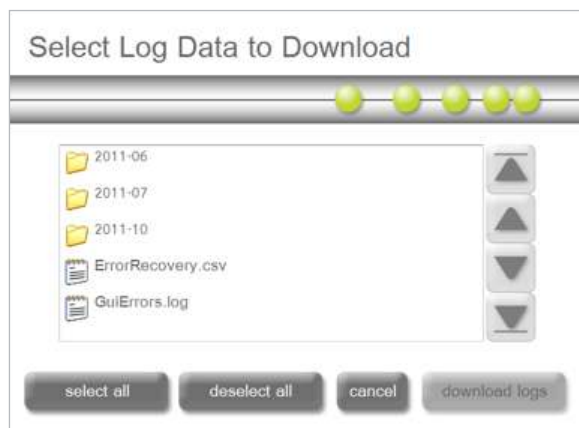
FIGURE 3.19: Troubleshooting screen



2. Press the **download log files** button.

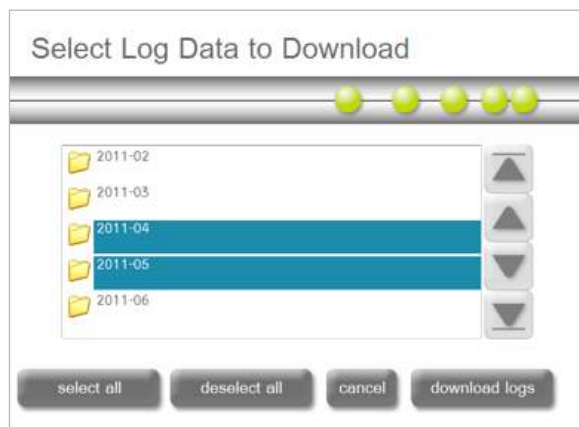
>>> The Select Log Data to Download screen appears (**Figure 3.20**).

FIGURE 3.20: Select Log Data to Download screen



3. Insert the memory stick into the USB port on the side 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 3.21: Select Log Data to Download screen



>>> When the files have successfully downloaded a message will appear (**Figure 3.22**).

FIGURE 3.22: Successful download message screen



5. Take the memory stick out of the Digital Analyzer and insert it into a computer. Email the log files to support@nanosttring.com. A NanoString Technical Support person will review the log files to help you determine any issues your instruments might be having.



NanoString Technologies, Inc.

530 Fairview Ave N
Suite 2000
Seattle, Washington 98109

CONTACT US

info@nanosttring.com
Tel: (888) 358-6266
Fax: (206) 378-6288
www.nanosttring.com

SALES CONTACTS

United States:	us.sales@nanosttring.com
Europe:	europe.sales@nanosttring.com
Japan:	japan.sales@nanosttring.com
Other Regions:	info@nanosttring.com

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