GeoMx® DSP
Instrument
User Manual

MAN-10152-04 for v3.1 software | November 2023

nanoString®

NanoString Technologies, Inc.
530 Fairview Ave N
Seattle, Washington 98109

www.nanostring.com

T:888.358.NANO (6266)
F: 206.378.6288
E: support@nanostring.com

Sales Contacts

United States: us.sales@nanostring.com
EMEA: europe.sales@nanostring.com
Asia Pacific & Japan: apac.sales@nanostring.com
Other Regions: info@nanostring.com

EU Authorized Representative

NanoString Technologies
Germany Gmbh
Birketweg 31
80639 Munich
Germany

UK Authorized Representative

NanoString Technologies
Europe Limited
11th Floor Whitefriars
Lewins Mead
Bristol BS1 2NT
United Kingdom

Rights, License, & Trademarks

Use

For research use only. Not for use in diagnostic procedures.

Intellectual Property Rights

This GeoMx® Digital Spatial Profiler (DSP) User Manual and its contents are the property of NanoString Technologies, Inc. ("NanoString"), and are intended solely for use by NanoString customers, for the purpose of operating the GeoMx DSP System. The GeoMx DSP System (including both its software and hardware components) and this User Guide and any other documentation provided to you by NanoString in connection therewith, are subject to patents, copyright, trade secret rights and other intellectual property rights owned by, or licensed to, NanoString. No part of the software or hardware may be reproduced, transmitted, transcribed, stored in a retrieval system, or translated into other languages without the prior written consent of NanoString. For a list of patents, see www.nanostring.com/company/patents.

Limited License

Subject to the terms and conditions of the GeoMx DSP System contained in the product quotation, NanoString grants you a limited, non-exclusive, non-transferable, non-sublicensable, research use only license to use the proprietary GeoMx DSP System only in accordance with the manual and other written instructions provided by NanoString. Except as expressly set forth in the terms and conditions, no right or license, whether express, implied or statutory, is granted by NanoString under any intellectual property right owned by, or licensed to, NanoString by virtue of the supply of the proprietary GeoMx DSP System. Without limiting the foregoing, no right or license, whether express, implied or statutory, is granted by NanoString to use the GeoMx DSP System with any third party product not supplied or licensed to you by NanoString or recommended for use by NanoString in a manual or other written instruction provided by NanoString.

Trademarks

NanoString, NanoString Technologies, the NanoString logo, GeoMx, CosMx, AtoMx and nCounter are trademarks or registered trademarks of NanoString Technologies, Inc., in the United States and/or other countries. All other trademarks and/or service marks not owned by NanoString that appear in this document are the property of their respective owners.

Open Source Software Licenses

Visit http://nanostring.com/geomx-oss for a list of open source software licenses used in the GeoMx DSP System.

Copyright

Table of Contents

Changes in this Revision

Changes in this manual revision include:

- Added information to support the use of the new Immuno-oncology Proteome Atlas (IPA) throughout the manual. **Please refer to the following page for a summary of key information and workflow changes for this assay. Note that GeoMx DSP software v3.1 is required to run the IPA assay.**
- Clarified that **New Data Collection** or **New/Continue Run** buttons are the ideal way to begin a collection. **Assist** (wrench), **Restart Data Collection Workflow** should only be used when the above buttons are not displayed.
- Added guidance on when to include or exclude the no-template control (NTC) in NGS-readout collections on page 31. Note that the NTC may not be in well A1.
- Added instructions to use Scan Templates, a new feature of software v3.1, on page 34.
- Added information to support third-party ROI selection using OME-XML files, in partnership with Visiopharm® AI-driven precision pathology software, on page 39.
- Updated GeoMx DSP hibernation protocol to flush both Buffer S fluidic lines on page 104.
- Added guidance to avoid overtightening the waste bottle cap, on page 108
- Removed guidance to keep a collection plate in the instrument at all times, since the software has been updated to resolve this issue.
- Updated figures and text for accuracy and clarity.

Key Considerations for the Immuno-oncology Proteome Atlas (IPA) with Pro Code Indices

NanoString's **Immuno-oncology Proteome Atlas (IPA)** covers 570+ protein targets across dozens of pathways and is the first GeoMx assay to use **Pro Code** i5 and i7 indices (sequences important in Next-Gen Sequencing (NGS)). Pro Code indices are 2 nucleotides longer than **Seq Code** indices, which are used in all other GeoMx NGS assays. For more information on i5 and i7 indices, please refer to the GeoMx DSP NGS Readout User Manual (MAN-10153).

When running a Pro Code assay such as the Immuno-oncology Proteome Atlas, please pay attention to Pro Code-specific instructions throughout the user manuals, summarized here:

- GeoMx DSP software v3.1 or later is required to run Pro Code assays such as IPA.
- The IPA probe mix is packaged as two tubes: core and module. The concentration of the module is different than NanoString's other Protein-NGS Assays with Seq Code indices, so the Antibody Working Solution is prepared differently. See details in the GeoMx DSP Manual Slide Preparation User Manual (MAN-10150) or Automated Slide Preparation User Manual (MAN-10151).
- Generally, assays with Pro Code indices such as IPA cannot be combined in the same plate or readout group as assays with Seq Code indices. Compatibility rules are detailed in the GeoMx DSP Instrument User Manual (MAN-10152). An exception is the Spatial Proteogenomic Assay in which RNA and Protein are collected from the same slide; refer to the GeoMx DSP Spatial Proteogenomic Assay User Manual (MAN-10158).
- In NGS library preparation, tags with Pro Code indices such as IPA are PCR-amplified with Pro Code primer plates, rather than Seq Code primer plates. Less DSP aspirate is required in the PCR amplification step for Pro Code assays compared to Seq Code assays (2 µL vs 4 µL). See details in the GeoMx DSP NGS Readout User Manual (MAN-10153). Note that 2 Pro Code primer plates are available (Y and Z), allowing multiplexing of up to 192 wells.
- Pro Code assays can generally be sequenced at less depth than Seq Code assays. See details in the GeoMx DSP NGS Readout User Manual (MAN-10153).
- At this time, Proteogenomic Assays that include IPA can only be processed on the standalone GeoMx NGS Pipeline software version 3.1, not on the GeoMx NGS Pipeline on DRAGEN via BaseSpace Sequencing Hub. Obtain GeoMx NGS Pipeline software version 3.1 and installation instructions at https://nanostring.box.com/v/GeoMxNGSPipeline. Refer to the GeoMx DSP Spatial Proteogenomic Assay User Manual (MAN-10158).
- There are no changes in the Data Analysis workflow for Pro Code assays.

Conventions

The following conventions are used in the GeoMx DSP user manuals and are described for your reference.

Bold text is typically used to highlight a specific button, keystroke, or menu option. It may also be used to highlight important text or terms.

Blue underlined text is typically used to highlight links and/or references to other sections of the manual. It may also be used to highlight references to other manuals or instructional material.

A gray box indicates general information that may be useful for improving assay performance. These notes aim to clarify other instructions or provide guidance to improve the efficiency of the assay workflow.

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

WARNING: This symbol 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.

For NGS readout: Content in blue boxes denotes steps or information specific to NGS readout of GeoMx DSP. Follow these instructions if using Illumina® NGS to read out GeoMx DSP counts.

For nCounter readout: Content in green boxes denotes steps or information specific to nCounter readout of GeoMx DSP. Follow these instructions if using nCounter® Pro, MAX/FLEX or SPRINT to read out GeoMx DSP counts.

GeoMx DSP Instrument Introduction

The GeoMx Digital Spatial Profiler is a benchtop instrument designed to spatially resolve and collect genomic information from distinct, user-defined regions of interest. The GeoMx DSP instrument is intended for Research Use Only. This **GeoMx DSP Instrument User Manual** is concerned exclusively with the operation of the GeoMx DSP instrument. Separate user manuals provide information on slide preparation, data readout by NGS or nCounter platform, and data analysis (see User Manuals and Resources on page 20) Please refer to training resources in NanoString University at https://university.nanostring.com and address questions or concerns to Support@nanostring.com.

Instrument Components

Figure 1: GeoMx DSP instrument exterior diagram

The GeoMx DSP instrument is a benchtop instrument and comes equipped with a 27″ color LED monitor (4K - 8MP, model: LG 27HJ712C-W), as well as a mouse and keyboard.

The front of the instrument has a USB port (USB-A 3.0) and a barcode scanner (Figure 1).

The main (upper) door opens to the GeoMx DSP instrument stage (Figure 2). The reagent bay (lower) door provides access to the buffer bottles and waste container .

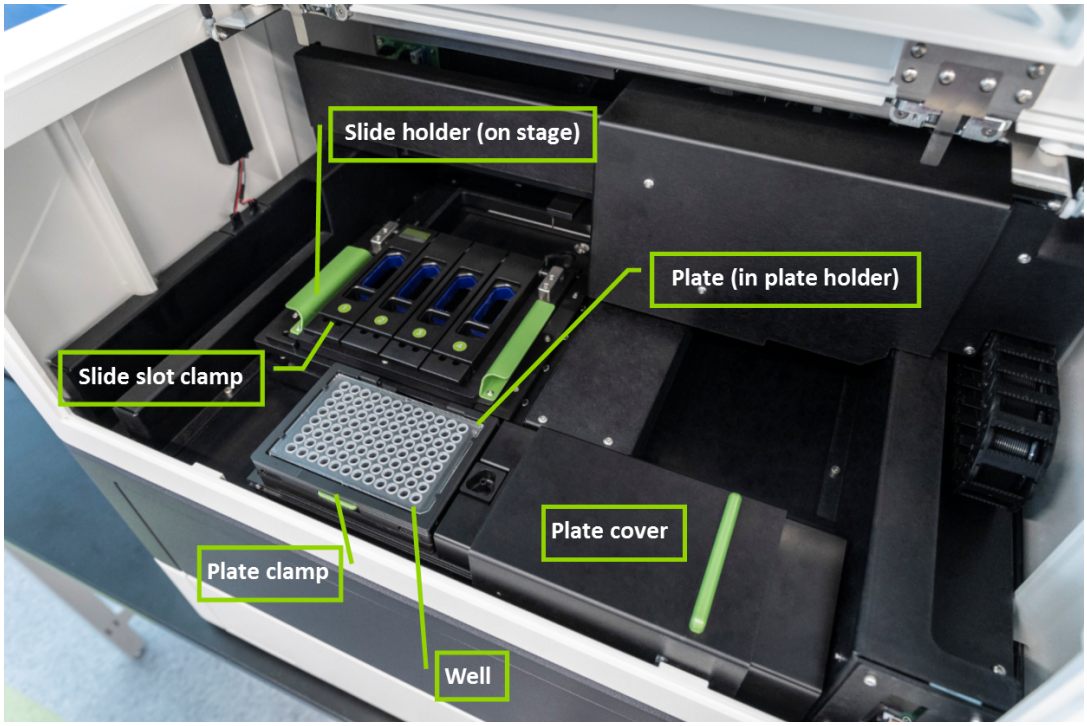


Figure 2: GeoMx DSP instrument interior diagram

To access the GeoMx DSP instrument stage, open the main (upper) door (Figure 2). On the stage, a collection plate is loaded into the plate holder and secured by the plate clamp. When loading a collection plate before your slides, the system will prompt you to slide the plate cover over the collection plate until loading is complete. Up to four slides can be loaded into individual slots on the slide holder, secured with the slide slot clamps, and covered with buffer. The slide holder is then placed onto the stage of the instrument.

Figure 3: GeoMx DSP instrument reagent bay diagram

The GeoMx DSP instrument requires the following reagent bottles in the reagent bay (Figure 3):

- **Buffer S**: 1 L bottles in reagent bay slots 1 and 4.
- **Buffer H**: 250 mL bottles in reagent bay slots 2 and 3.

In addition, the **waste bottle**, with adequate space to collect waste, must be present in the position to the right of the reagent bottles.

> **IMPORTANT:** Always use the **Change Reagents wizard** and exchange full bottles. DO NOT top off bottles, or exchange bottles without the Change Reagents wizard.

For information on replacing reagent bottles, see GeoMx DSP Instrument Reagents on page 98.

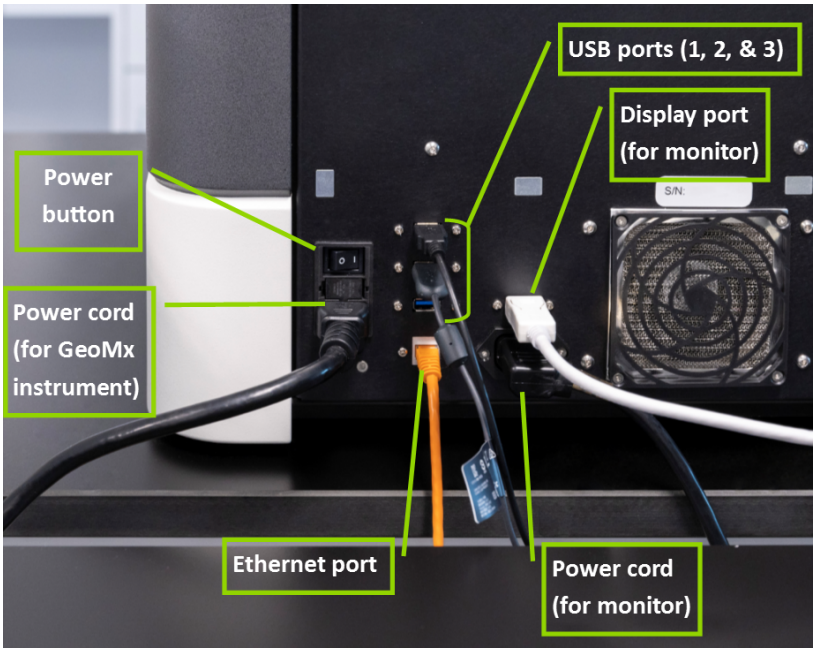


Figure 4: Diagram of back of GeoMx DSP instrument

The back of the GeoMx DSP instrument (Figure 4) has the following ports:

- An ethernet connection: CAT 6 Network Port with RJ45 connector.
- Three USB connections: USB-A 3.0.
- A power input for the instrument: Power in 100 – 240 VAC, 50/60 Hz, connector C14.
- A power output for the monitor: Power out (same as Power in), connector C13.
- A display port for the monitor.

Instrument Specifications

The GeoMx DSP system is a single instrument, intended for Research Use Only. The necessary cables and a waste bottle are included with each instrument. **Do not dispose of the waste bottle.** All other consumables and reagents may be purchased separately.

Table 1: GeoMx instrument specifications

Instrument Specifications	
Weight	210 lb (95.3 kg)
Dimensions (W x D x H)	30 in (760 cm) W x 29 in (73 cm) D x 24 in (61 cm) H
Power Requirements	100 - 240 VAC, 50/60 Hz, 440 VA
Fuse	10 A, 250 V Slow Blow
Altitude	≤2000 m
Pollution degree	2
Overvoltage category	II
Room Temperature	18–28 °C
Room Humidity	30–80% relative humidity (non-condensing)
NOTE: The combination of high temperature and high humidity (even within the ranges stated above) may create condensation on certain internal components of the instrument which can cause damage to the system. If the environment is humid, or can become humid seasonally, it is recommended to place the instrument in an air-conditioned or lower-temperature room and/or run a dehumidifier nearby.	

The instrument is installed with an uninterruptible power supply or UPS provided by NanoString. The UPS is designed to support the equipment it is connected to for a short duration, until the utility power has been restored or backup power (e.g., a generator) has come online. For the GeoMx DSP instrument alone, a UPS with new batteries is expected to run for about 50 minutes. For the GeoMx DSP instrument plus an Auxiliary Server, a UPS with new batteries is expected to run for about 30 minutes. For technical specifications, please see the manufacturer's information specific to the model and serial number of your UPS.

Fluorescence Channel Specifications

The wavelengths and excitation and emission information are listed below for each of the GeoMx DSP channels.

Table 2: GeoMx fluorescence channel specifications

Channel	Excitation (peak/bandwidth)	Emission (peak/bandwidth)
FITC / 525 nm	480 / 28 nm	516 / 23 nm
Cy3 / 568 nm	538 / 19 nm	564 / 15 nm
Texas Red / 615 nm	588 / 19 nm	623 / 30 nm
Cy5 / 666 nm	645 / 19 nm	683 / 30 nm

Installing and Moving

- Indoor use only. Avoid locating in direct sunlight.
- The GeoMx DSP instrument may only be installed or moved by appropriately trained personnel. Contact NanoString for installation or relocation of the instrument. See Safety Information (English) on page 16.
- Place the instrument on a flat, stable surface with access to a dedicated power supply.
- Ensure adequate clearance on the backside of the instrument in order to access power controls and allow at least 3 inches for ventilation.
- Ensure adequate clearance on the front of the instrument to allow opening of the main instrument door and the reagent bay door.
- Avoid locating near other equipment that may cause vibration, or near large electrical equipment that may cause interference from noise and/or voltage fluctuation.
- Consult your laboratory guidelines and local regulations for information on reducing hazards associated with the transport, disposal, or removal of an instrument from use. There are no hazards unique to the GeoMx DSP instrument that require additional instruction in this manual, however, some general safety guidelines are provided in Safety Information (English) on page 16 and in Disposal of Electronic Equipment on page 15.

Product Use Limitations

The GeoMx DSP system is intended for Research Use Only. Not for use in diagnostic procedures.

Disposal of Electronic Equipment

Review 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 the WEEE directive, please contact your NanoString representative 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 Information (English)

The GeoMx DSP instrument may only be operated by appropriately trained, professional users for Research Use Only. NanoString recommends that all users read and understand this manual prior to attempting to operate the system. Keep this manual in close proximity to the instrument for easy access to instructions and safety information.

If the GeoMx DSP instrument is used in a manner not specified by NanoString, the protection provided by the equipment may be impaired. Failure to comply with the instructions in this manual may pose a dangerous risk to the operator and void the manufacturer's warranty.

Do not attempt to disassemble the equipment. The GeoMx DSP instrument 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 and malfunction, and will void the manufacturer's warranty. Do not replace detachable main supply cords by inadequately rated cords.

WARNING: Do not attempt to install, move, or perform maintenance on the instrument. Always contact NanoString for instruction before installing or moving the instrument. If it is necessary to move equipment, use extreme caution when lifting items that weigh more than 50 pounds (such as the GeoMx DSP instrument). Use two or more people to lift the load.

WARNING: Do not use this device in close proximity to sources of strong electromagnetic radiation or vibration, as these may interfere with proper operation.

WARNING: Operate the system using only NanoString reagents in accordance with their indications for use. Ensure that all consumables are properly inserted prior to starting a run.

WARNING: Connect the instrument power supply to a properly grounded receptacle with adequate voltage and current (see Instrument Specifications on page 13).

Caution Symbols

ELECTRICAL HAZARD: Do not attempt to disassemble the instrument at any time. An electric shock can occur if the instrument is operated without its outer case.

Consignes de Sécurité (Français)

Le GeoMx DSP instrument ne peut être utilisé que par une personne ayant reçu une formation professionnelle appropriée. NanoString recommande que tous les utilisateurs lisent et comprennent ce manuel avant de tenter de faire fonctionner le système. Conservez ce manuel à proximité de l'instrument pour faciliter l'accès aux instructions et consignes de sécurité.

Si le GeoMx instrument n'est pas utilisé de la façon spécifiée par Nanostring, la protection fournie par l'équipement pourrait être altérée. Le non- respect des instructions de ce manuel peut présenter un risque dangereux pour l'opérateur et peut annuler la garantie du fabricant.

Ne pas tenter de démonter l'équipement. Le GeoMx DSP instrument contient des pièces non réparables par l'utilisateur. Un réparateur agrée par le fabricant doit faire les réparations. Ne pas modifier les pièces sous peine de provoquer un incendie ou un mauvais fonctionnement, de plus cela annulera la garantie du fabricant. Ne pas remplacer les cordons d'alimentation amovibles par des cordons insuffisamment évalués.

⚠ **ATTENTION:** Ne pas tenter d'installer, de déplacer ou d'effectuer l'entretien de l'instrument. Toujours contacter NanoString pour instruction avant d'installer ou le déplacer l'instrument. S'il est nécessaire de déplacer l'instrument, utiliser une extrême prudence pour soulever des objets pesant plus de 23 kilogrammes. Pour soulever une charge plus lourde que 23 kilogrammes (comme pour l'instrument GeoMx DSP), utiliser au moins deux personnes pour soulever la charge.

⚠ **ATTENTION:** Ne pas utiliser cet appareil à proximité de sources à fortes radiations électromagnétiques ou vibrations, car elles peuvent interférer avec le bon fonctionnement de l'appareil.

⚠ **ATTENTION:** Faire fonctionner le système en utilisant uniquement des réactifs NanoString conformément à leurs guides d'utilisation. Assurez-vous que tous les consommables soient correctement insérés avant de commencer une opérat

⚠ **ATTENTION:** Branchez l'alimentation de l'appareil électrique à une prise correctement mise à la terre avec une tension et un courant adéquat (voir les spécifications de l'instrument).

Définitions des Étiquettes de Sécurité

RISQUE ÉLECTRIQUE: Ne jamais tenter de démonter l'appareil. Un choc électrique peut se produire si l'appareil est utilisé sans son enveloppe extérieure. Débranchez l'appareil de la source d'alimentation avant de remplacer le fi ltre du ventilateur.

GeoMx DSP Workflow

The GeoMx Digital Spatial Profiler (DSP) is a novel platform developed by NanoString. Antibody or nucleic acid probes are coupled to photocleavable oligonucleotide tags. After probes hybridize to targets in slide-mounted tissue sections, the oligonucleotide tags are released from discrete regions of the tissue via UV exposure. Released tags are quantified by nCounter technology or Illumina Next Generation Sequencing (NGS). Counts are mapped back to tissue location, yielding a spatially resolved digital profile of analyte abundance (Figure 5).

- **Day 1: Slide Staining.** Prepare slides and incubate biological targets with UV-cleavable probes. Prepare manually or using the BOND RX/RX^{m} Fully Automated IHC/ISH Stainer from Leica Biosystems®.
- **Day 2: Process Slides on GeoMx DSP.** Load prepared slides into the GeoMx DSP instrument. Slides are scanned to capture fluorescent images used to select regions of interest (ROIs). ROIs may be segmented into discrete compartments or areas of illumination (AOI). The instrument collects UV-cleaved oligos from the AOIs into the wells of a collection plate.

For NGS readout:	For nCounter readout:
Day 3: Transfer the collected aspirates to a PCR plate and perform **Library Prep** with Seq Code or Pro Code primers. Pool and purify the products, then **Sequence** on an Illumina NGS instrument. **Day 4**: Process FASTQ sequencing files into digital count conversion (DCC) files using **GeoMx NGS Pipeline** with NanoString's standalone software or Illumina's DRAGEN™ platform accessed via BaseSpace™ Sequence Hub (cloud) or NextSeq 1000/2000 (local).	**Day 2, continued**: Transfer the collected aspirates to a hybridization plate along with GeoMx Hyb Code reagents. Hybridization occurs overnight. **Day 3**: Pool wells and **Process on an nCounter Pro or MAX/FLEX Analysis System or SPRINT Profiler** to generate reporter count conversion (RCC) files.

- **Day 4 or 5: Upload counts** to GeoMx DSP and **create a study** in the Data Analysis Suite. Perform quality-control checks and data analysis, and generate analysis plots.

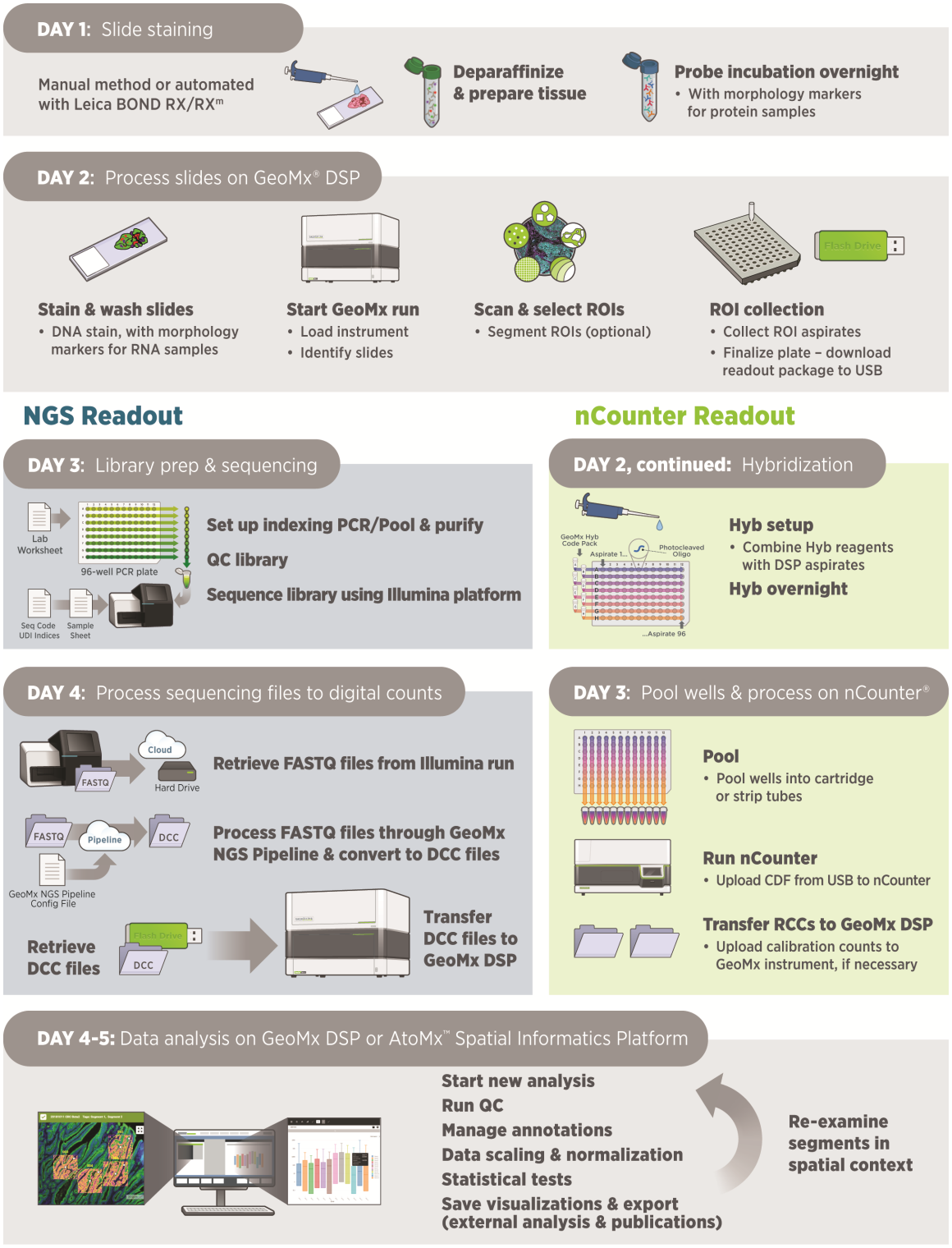


Figure 5: GeoMx DSP workflow summary

User Manuals and Resources

The GeoMx DSP workflow is divided into the following user manuals:

Workflow Step 1	GeoMx DSP Manual Slide Preparation User Manual MAN-10150 GeoMx DSP Automated Slide Preparation User Manual MAN-10151	
Workflow Step 2	GeoMx DSP Instrument User Manual MAN-10152	
Workflow Step 3	**For NGS readout:** GeoMx DSP NGS Readout User Manual MAN-10153	**For nCounter readout:** GeoMx DSP nCounter Readout User Manual MAN-10089
Workflow Step 4	GeoMx DSP Data Analysis User Manual MAN-10154	

User manuals and other documents can be found online in the NanoString University Document Library at https://university.nanostring.com. Instrument and workflow training courses are also available in NanoString University.

For NGS readout:
For documentation specific to the Illumina platform, see https://support.illumina.com.

For nCounter readout:
For documentation specific to the nCounter Pro, MAX/FLEX, and SPRINT instruments, visit the NanoString University Document Library at https://university.nanostring.com.

For the GeoMx DSP Spatial Proteogenomic Protocol (RNA and Protein on the same slide), please refer to the GeoMx DSP Spatial Proteogenomic Assay User Manual (MAN-10158).

GeoMx DSP Control Center

The GeoMx DSP Control Center is the main interface of the GeoMx DSP software.

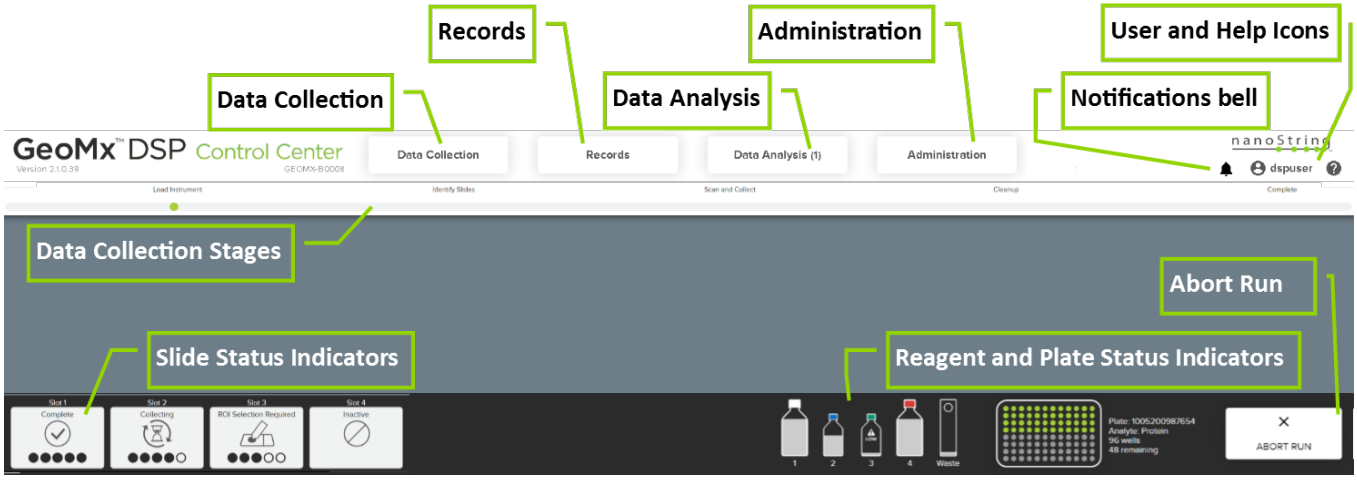


Figure 6: GeoMx DSP Control Center diagram

By clicking the **Data Collection** button in the GeoMx DSP Control Center header (Figure 6), you can begin a new run or upload counts or calibration files. The **Records** button allows you to create slide records and scan parameters and to add scans to the Data Analysis Queue. Select the **Data Analysis** button to build a study from the queue or open a study. The **Administration** button opens a menu of administrative settings.

The **Data Collection Stages**, depicted just below the header when **Data Collection** is selected, provide you with a visual orientation of the progress of your run.

The **Notifications bell** will alert you to errors in archiving, the status of readout file upload, and the status of a data analysis build. These notifications will remain until you click the bell; once seen, they will persist for 24 hours or until closed with the x (archiving errors may regenerate hourly).

Slide and **Plate Status** indicators in the footer indicate the current state of the slides and plate, respectively.

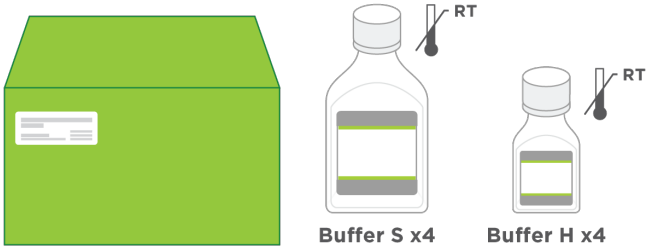
Reagent Status indicators depict the reagent and waste bottle levels. A locally logged-in user may use the **Abort Run** button, if necessary, to halt the run.

GeoMx DSP Run

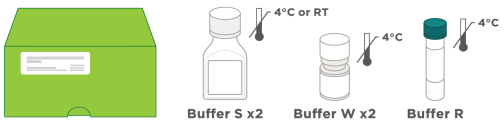
Reagents and Consumables

In addition to the reagents in the kits listed below, you will need **lint-free wipes**, **distilled water (dH_2O)**, and **70% ethanol** for cleaning the slides before the run.

GeoMx Instrument Buffer Kit



GeoMx Slide Prep Kit (only Buffer S is needed from this kit)



GeoMx RNA Slide Prep Kit

GeoMx Protein Slide Prep Kit

GeoMx DSP Collection Plates (not pictured)

Start a New Run

It is recommended to reboot (shut down and restart) the instrument before starting a new run. Then, click the button **New Data Collection**, if displayed, or hover over **Data Collection** (Figure 7) and select **New/Continue Run**.

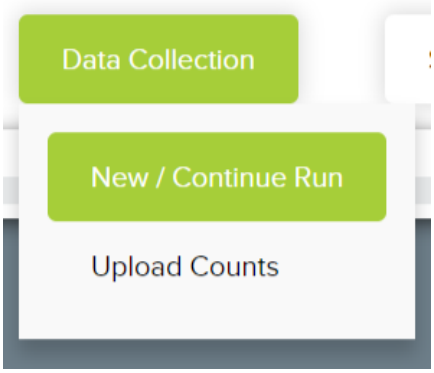


Figure 7: New/Continue Run button

If a Data Collection run is in progress, clicking **New/Continue Run** will take you to the current step in the run.

Load the slides into the slide holder

1. Remove the clean slide holder from the instrument and open the slide slot clamps. Inspect the holder to ensure there is no residue from a previous run. Manually move the spring clamp to ensure that it moves freely and is not blocked by debris. Use a lint-free wipe and **dH_20** to clean the slide holder, if needed. See Cleaning the Slide Holder on page 103 for cleaning guidelines.
2. Load slides (through step 5) one at a time to minimize exposure to air.
   - Clean the bottom of your slide with a lint-free wipe and **70% ethanol**.
   - Inspect your slide for debris and residue, such as material from the hydrophobic pen if used during slide preparation.

     IMPORTANT: Slide labels must not be positioned beyond the frosted portion of the slide or under the gasket of the slide clamp. Avoid using slides with colored or blank labels, which may not be detected by the imaging system. Add a dark mark to the slide label area using a permanent lab pen to improve slide detection if needed.

3. Place your slide in the slide holder, face up, label toward slide holder clamp opening (Figure 8). Ensure that the slide is evenly seated in the slide holder slot.
4. Lower the slide holder clamp (Figure 9).
   - The slide label should be visible in the rectangular window above the green slide slot number.
   - **Tissue may not extend beyond the gasket boundary.**
   - Clean the bottom of the slide again, if needed, with a lint-free wipe and **70% ethanol**.

Figure 8: Loading the slide holder

Figure 9: Lowering the slide holder clamps

IMPORTANT: Ensure that there is no tissue, slide label, or other material between the gasket and the slide. Failure to clear material can affect gasket sealing, causing leakage and instrument damage.

5. Add **6 mL Buffer S** onto each slide (Figure 10). Use Buffer S from the Slide Preparation Kit.

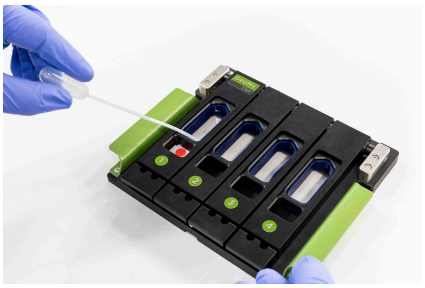


Figure 10: Adding buffer

IMPORTANT: Do not leave your slides exposed to air or light. Keep covered with buffer and shielded from light at all times.

6. Record the location of each slide in the slide holder.

Set up the GeoMx DSP instrument

The prompts may appear in a different order than that listed here.

Replace Plate?

This screen appears when the system detects that a collection plate is already loaded on the instrument.

Select **Yes** to replace the collection plate. Select **No** to use the collection plate currently loaded on the GeoMx DSP instrument. Select **Next.**

- **Completely used collection plate:** if the selected plate is already full, you will be prompted to use a different plate.
- **Partially used collection plate:** if the selected plate is only partially full, you may choose to use the remaining wells for the present run, as long as the present run is compatible with the collection already in the plate (see compatibility rules on the next page). Collection will be set to use the next available well or row on the plate.

Open the Door

Open the instrument door when prompted. Once the door is open (Figure 11), the system will display the next step.

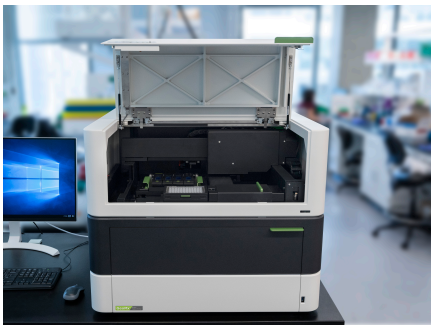


Figure 11: Open the GeoMx instrument door when prompted

If the door seems to be locked when it should be unlocked, push down lightly on the door, then begin to pull it open. It should then unlatch and open.

Identify Plate

Each NanoString collection plate has a barcode along one side. This barcode is used to track the plate and its aspirates throughout the DSP workflow.

Scan the plate's barcode by holding it at a 45° angle to the right of the scanner and moving the plate between 2-6 inches from the instrument. The barcode will auto-fill when read. Alternatively, use a handheld scanner (not provided) or enter the barcode manually.

- If the plate's barcode has not been previously read by the instrument, it is assumed to be a new plate.
- If the plate's barcode is recognized and the plate has no eligible remaining wells to fill, you will be prompted to use a different plate.
- If the plate's barcode is recognized and it was previously partially-filled, collection will begin in the next available well or row of the plate:
    - If the previous collection was finalized, a different readout group must be used. Collection will begin in the next available row of the plate.
    - If the previous collection was not finalized, the same readout group must be used. Collection will begin in the next available well of the plate, unless the new collection is with a different kit configuration, in which case it will begin in the next available row.
    - Some combinations of assays are not allowed in one plate (Table 3). If an incompatible combination is selected at scan set-up, the instrument will present an error message.

Table 3: Compatibility of multiple assays in one plate

Combination in one plate	Allowed?
Combinations of single analyte assays (RNA or Protein) which use only Seq Code indices (i.e., **no** Immuno-oncology Proteome Atlas)	Yes
Single analyte assays using Pro Code indices (Immuno-oncology Proteome Atlas) **with** single analyte assays using Seq Code indices (all other Protein-NGS panels)	No
Single analyte assays (RNA or Protein) **with** mixed analyte assays (RNA + Protein)	No
Mixed analyte assays using Pro Code indices (Immuno-oncology Proteome Atlas) **with** mixed analyte assays using Seq Code indices (all other Protein-NGS panels)	No

- Invalid barcode: if the barcode is not in a valid format (13 digits), you will be prompted to try re-entering the barcode or to use a different collection plate.

For NGS readout:
Select **Illumina** from the **Downstream Counting Platform** dropdown field.
Choose the **Readout Group**. This is the selection of plates you want grouped together. Choose from a previously-established group or create a new group. Do not use special characters such as $! / in readout group names.

For nCounter readout:
The row into which aspirates are collected must match the Hyb Code used later in the workflow. Aspirates collected into row A must be hybridized with Hyb Code A, row B with Hyb Code B, etc. Therefore, consider the Hyb Code reagents you have on hand when planning your collection.

Click **Next**.

Insert the Collection Plate

1. Open the plate holder clamp.
2. Insert the collection plate onto the plate holder (Figure 12).

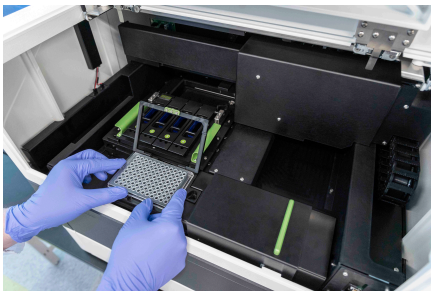


Figure 12: Insert the collection plate onto the plate holder when prompted

3. Fasten the clamp (Figure 13).
4. Once the clamp is fastened, the system will display the next step.

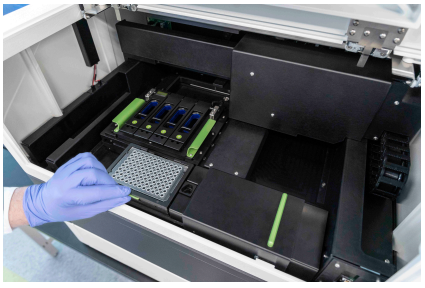


Figure 13: Fasten the clamp on the collection plate

Cover/Uncover the collection plate

This prompt will appear if your collection plate was loaded before your slides. Covering the collection plate (Figure 14) as you load the slide holder onto the stage prevents contamination from spills or debris.

Once the plate cover is moved (according to the prompt), the system will display the next step.

The plate must be uncovered for the instrument run.

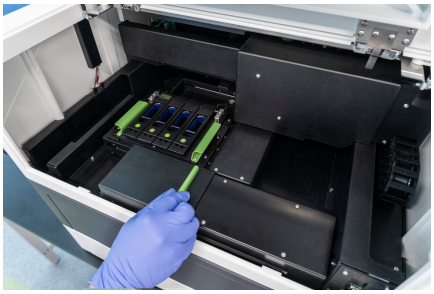


Figure 14: Cover the collection plate when prompted

Insert the Slide Holder

Find information on loading the slides into the slide holder on page 23.

- Load the slide holder onto the stage of the instrument (Figure 15) when prompted.
- Inspect visually and by touching to ensure that the slide holder fits evenly in the frame of the stage.
- The green slide numbers should be closest to the front of the machine.

Figure 15: Insert the slide holder onto the stage of the instrument when prompted

Select **Next**.

For information on removing the slide holder, see Removing the Slide Holder on page 62.

Close the Door

When prompted to close the door, first ensure that the plate cover is pushed all the way to the right. Once the door is closed, the system will confirm that the plate cover is out of the way and lock the door.

The plate must be uncovered for the instrument run.

The system will detect the necessary components for the run. It will then proceed to Identify Slides on page 29.

Identify Slides

Once the necessary components for the run have been detected, the system will commence **Slide Identification** by taking a low-magnification image of each loaded slide (Figure 16).

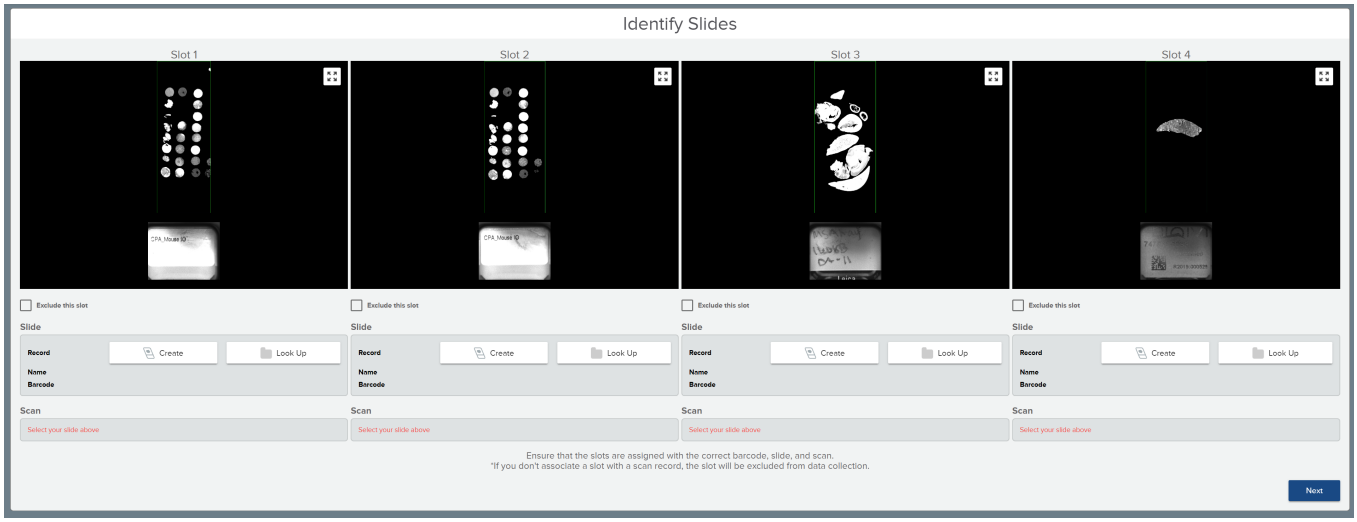


Figure 16: Identify Slides stage in GeoMx DSP run workflow

Verify or complete the fields underneath each slide image

You can link slides to any slide records and scan parameters created earlier. See Create Slides and Scans outside GeoMx DSP Workflow on page 68.

- **Exclude this slot:** Check this box to exclude the slide from further processing.
- **Slide Record**: contains the metadata for the slide. Click **Create** or **Look Up:**

  Create:

  - From the **Identify Slides** window, select **Create**.
  - Open or create a new parent folder from the **Navigation** window.
  - The **Creating new slide record** window opens to the right of the Navigation window. Fill in:
    - Slide label (the unique identifier for this physical slide)
    - Barcode (optional)
    - Description (optional)
  - Click **Save Slide**.

Look up:

- From the **Identify Slides** window, select **Look up**.
- Select a parent folder from the **Navigation** window.
- **Select a slide**. The slide record will appear to the right of the Navigation window. Verify the information in the slide record.
- Click the **Select Slide** button.

- **Barcode**: auto-populates with the slide record barcode (if entered) when linked to a slide record.
- **Scan**: contains the parameters for the scan.
  - If you established scan parameters in advance, they will appear in the dropdown menu; select the desired set of scan parameters.
  - If no scan parameters have been made, select **Create New Scan** from the dropdown menu (see Create New Scan on page 30).

Create New Scan

Scan parameters define the settings for the scan. When you create a new set of scan parameters, the Scan Configuration window opens (Figure 17). Load a **scan template** containing pre-filled parameters, or enter parameters manually. See Scan Templates on page 34.

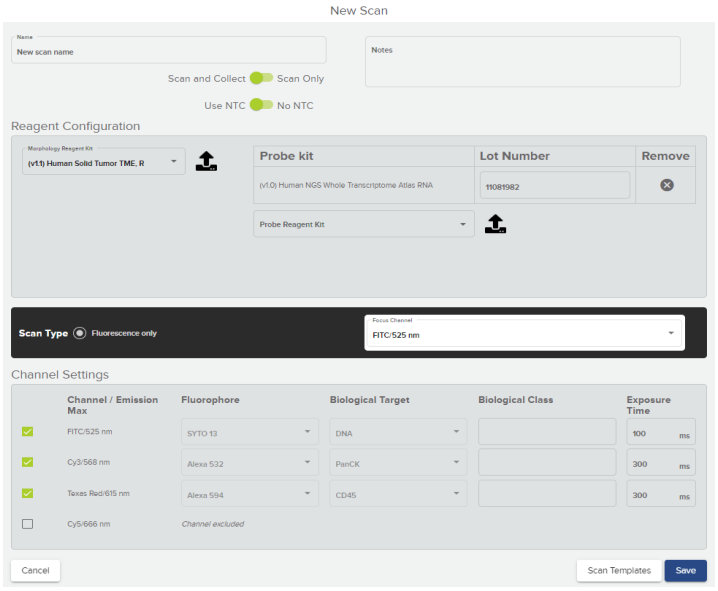


Figure 17: Scan parameters in Scan Configuration window

1. **Enter a Name** for this scan. This should be a unique identifier for this instance of scanning, therefore it is recommended to include the date or a number sequence. Scan names should not have leading or trailing spaces.

2. Toggle to select **Scan and Collect** or **Scan Only**.

   Scan Only mode enables slide scanning and ROI selection without collection. ROIs selected on a Scan Only slide can be transferred to a Scan and Collect slide at another time using the ROI Transfer function (see ROI Transfer on page 56). This feature is useful when evaluating tissue staining conditions prior to the experimental run, or practicing/discussing ROI selection prior to ROI collection, for instance.

   See Scan Only on page 38.

3. **Use NTC**: Once a probe kit is selected which designates this scan as NGS-readout, the NTC (no-template control) option is enabled.

   An NTC helps with troubleshooting data or library prep issues that may occur. Without an NTC, library QC and initial data QC are limited, and NanoString's ability to troubleshoot problems is limited. **Only labs in which the GeoMx NGS workflow is running with proven success should consider collecting from slides without an NTC.**

   If any scan in the collection is set to **Use NTC**, one NTC well will be designated in the plate. Depending on scan settings, the NTC well may not be well A1. Check the Lab Worksheet in the readout package to know which well is the NTC. Even if multiple scans are set to Use NTC, only one well is designated.

4. **Select Reagent Configuration files.**

   GeoMx DSP probe kit configuration (.pkc) files associate GeoMx readout barcodes with RNA and Protein assay targets. Morphology Kit configuration (.mkc) files provide visualization target information to the GeoMx DSP software.

   - Configuration files are not pre-loaded on the instrument and must be transferred using a USB drive or remote access over Chrome. Find the files for the probe kits used on your slides at www.nanostring.com/dspconfigfiles. Save these files to a USB drive. **Unzip** files before uploading to the instrument. **NanoString recommends uploading new .pkc files under Kit Management in the Administration menu** (see Kit Management on page 89). Once uploaded to the instrument, they are available for selection in the Scan Configuration window.
   - Select the **Morphology Reagent Kit** used in preparing the slide from the dropdown. If not already loaded on the instrument, select the **upload** button to the right of this field to upload the appropriate **unzipped** configuration file from your USB drive (see above). See Example Scan Parameters on page 70 for GeoMx Solid Tumor TME and Melanoma Morphology parameters.
   - Select the probe kits used in preparing the slide from the **Probe Reagent Kit** dropdown. (If setting up in Scan Only mode, do not select a Probe Kit.) The Core Kit must be selected before the compatible Module Kits will be available in the dropdown. Select the **upload** button to upload new configuration

files from your USB drive, if needed. If newly uploaded files do not appear in the dropdown, exit the Scan Configuration window, then re-open.

IMPORTANT: Please note that software v3.1 is required to run the Immuno-oncology Proteome Atlas.

- To collect from proteogenomic assay (RNA + Protein) slides, select the RNA and Protein .pkc files according to the kits used. Do not run a "mixed analyte" (RNA + Protein) slide in the same collection with a "single analyte" (RNA or Protein) slide.
- Enter the lot number of the probe kit(s). Your selected probe kits are listed to the right of the upload buttons.
- Uploaded probe kit configuration files can be activated and inactivated under **Kit Management** in the **Administration** menu (see Kit Management on page 89).

Ensure you have uploaded the correct configuration files for the core and each module used during slide preparation. Note the following:

- **Failure to select a Core probe kit** in the Probe Reagent Kit field will result in Scan Only mode **(no aspirate collection).**
- If using **a custom panel**, NanoString Bioinformatics will provide a custom .pkc file. Upload this file to the GeoMx DSP, then select it from the Probe Reagent Kit dropdown menu, along with any other probe kits in use in this collection.
- To **change the .pkc file** associated with a slide after collection is complete, see Changing a Probe Kit Configuration File After Collection on page 71.
- If you do not see the file you need, contact Support@nanostring.com for assistance.

For nCounter readout:

Do not combine slides with nCounter-RNA assays and slides with nCounter-Protein assays in the same collection.
Protein: If a protein module added to the list shares the same Probe R space as another module on the list, a warning will appear. Proceeding poses the risk of losing data from both modules. To enable collection with both modules, upload and select a Substitute Probe R configuration file at this step, and use Substitute Probe R in the nCounter readout of the assay. See Appendix IV of the GeoMx DSP Manual Slide Preparation User Manual (MAN-10150) for more information about Probe R.
RNA: If adding custom RNA targets to the RNA Immune Pathways Panel, only one custom RNA module can be added per scan.

5. Select the appropriate **Scan Type** and **Focus Channel** for your slide. The scan type option will be set to **Fluorescence** only. The focus channel selected must have abundant signal. **FITC/525 nm** (default for nuclear stain SYTO 13) is typically used.
6. Verify the **Channel Settings** fields. Ensure all channels you wish to use in the scan have been checked. If desired, adjust the value in the **Exposure Time** field, based on your empirical testing. If desired, adjust the name in the **Biological Class** field.
7. Fill in the custom morphology field (if applicable) to populate custom fluorescence exposure settings.
8. If desired, save the scan configuration as a template by clicking **Scan Templates**, then **Save** from the dropdown menu. You'll be prompted to name the template. (See Scan Templates on page 34.)
9. Select **Save** on the Scan Configuration window.
10. Select **Next**. The system will proceed to the **Review Tissue Detection** window – see Scan Slides on page 36.

Scan Templates

With software v3.1, users have the ability to save scan configurations as scan templates, to reduce the time needed to set up new scans and minimize the risk of error in entering scan configuration details. Saved scan templates are available to all instrument users.

To create a scan template from an existing scan's configuration,

1. Open the **Scan Configuration** window by clicking **Scan Parameters** on the scan's card in the gallery (Figure 18).
2. Click **Scan Templates**, then **Save** (Figure 19).

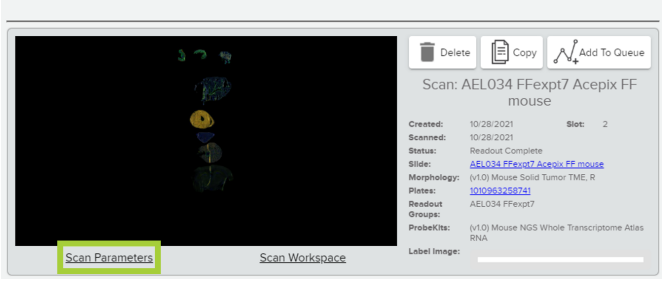


Figure 18: Open Scan Parameters

3. Enter a template name at the prompt and click **Save**.
4. The template is saved in the folder **Scan Templates** accessible at the top of the Navigation pane (Figure 20). To use it, see instructions on next page.

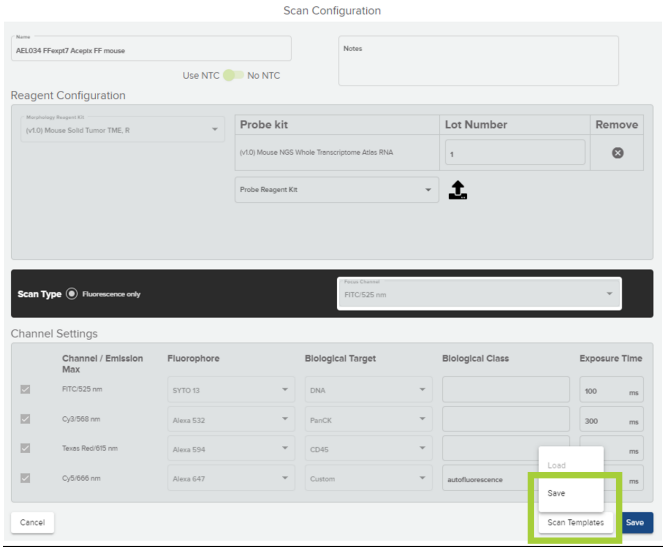


Figure 19: Save configuration as scan template

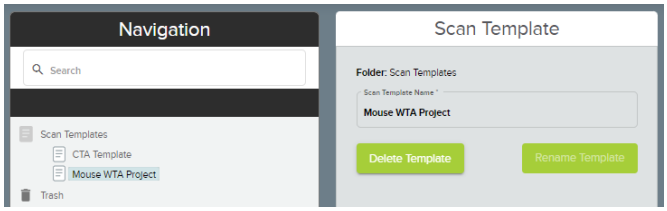


Figure 20: Scan templates in Navigation pane

To create a scan template using a new configuration,

1. Select a slide from the Navigation pane or create a new slide record (see Create a New Slide Record on page 68), then click **Add Scan** (Figure 21).
2. The Scan Configuration window opens (Figure 22). Enter the parameters to make up the template. (Values can be changed when loading and using the template.)
3. Click **Scan Templates**, then **Save** (Figure 22).

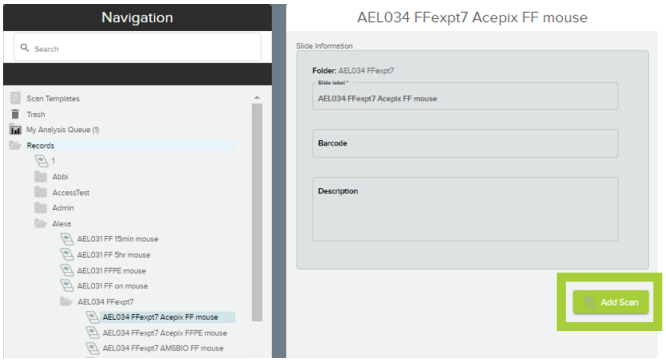


Figure 21: Add scan button

4. Enter a template name at the prompt and click **Save**.
5. The template is saved in the folder **Scan Templates**, accessible at the top of the Navigation pane (Figure 20). To use it, see below.

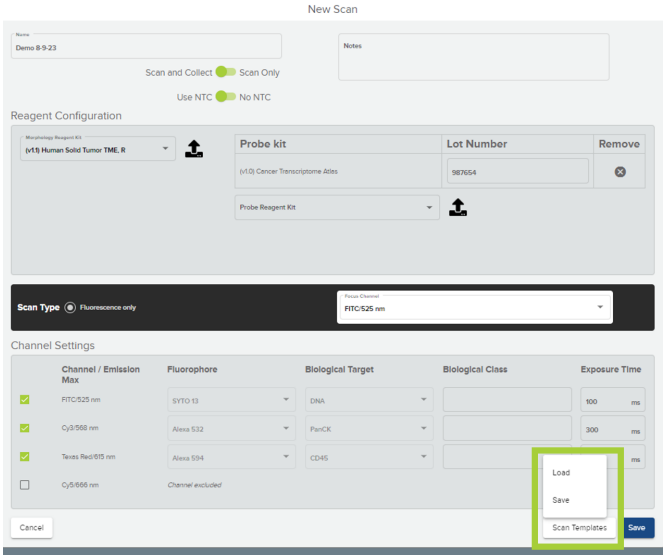


Figure 22: Save configuration as scan template.

To use a scan template when setting up a scan,

1. When the Scan Configuration window opens, select **Scan Templates**, then **Load** (Figure 22).
2. Select the scan template of interest from the dropdown menu. Click **OK**.
3. The parameters of the template load in the Scan Configuration window. If needed, parameters can be changed from the template's values.

Delete or **Rename** a scan template by clicking on the buttons on the Scan Template pane (Figure 20).

Scan Slides

Before the system's 20x scan, it checks the tissue area. You may accept the default parameters automatically detected by the system, or customize them.

Define Scan Area

The scan area result is displayed (Figure 23). Use the tools in this window to select *only* the tissue on the slide. This minimizes scan time and avoids unnecessary consumption of disk space. Areas within green boundary lines will be scanned. To adjust:

- **Zoom in** and **out** using the scroll wheel on your mouse or a pinch-zoom movement on your touch screen or touch pad.
- Adjust the scan area using the **X- and Y- sliders** to define the area you would like to analyze.
- Use the **Minimum Tissue** slider to filter out particles from the image. Sliding it too far may cause small areas of tissue to be excluded from the image.
- Use the **Sensitivity** slider to adjust the intensity at which the instrument identifies tissue.

IMPORTANT: The minimum scan area is 1.5 mm x 1.5 mm.

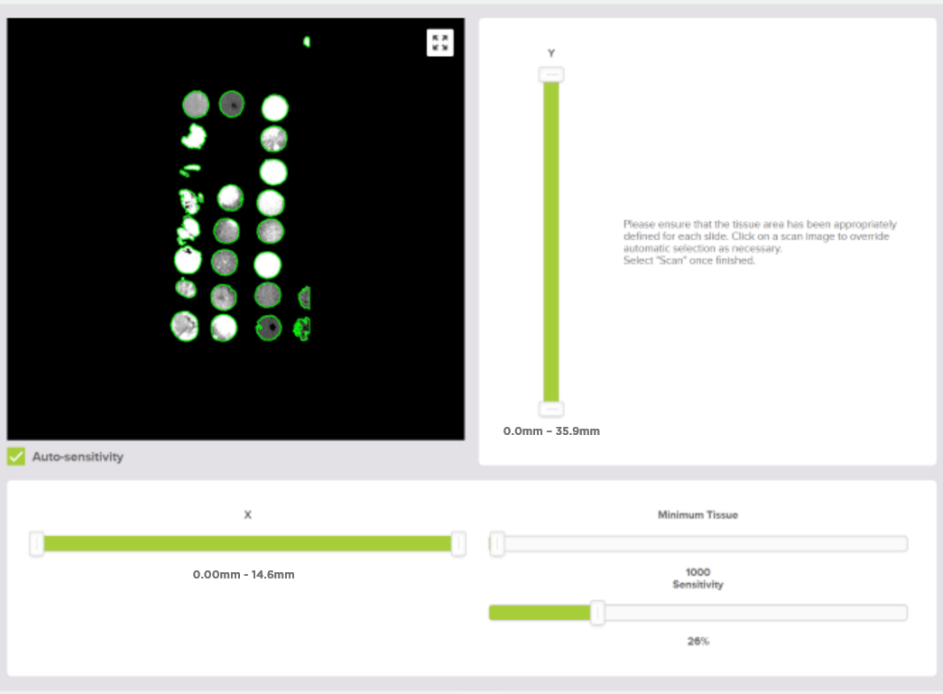


Figure 23: Scan area definition adjustment

Click through all slides using the **Previous Slide** or **Next Slide** buttons.

IMPORTANT: Inspect slides and settings carefully before proceeding, as settings cannot be modified once scanning begins.

When you have completed defining the scan area for all slides, select the **Scan** button.

The system will begin a high-magnification scan of each slide, using the defined tissue area and the scan parameters specified in the scan record (Figure 24).

- Once the entire 20x scan is complete for a slide, you may zoom in, zoom out, pan, and switch to full-screen mode within each slide view.
- Once scanning of a slide is complete, the **Edit ROIs** button underneath the image will appear.
- Click the **Edit ROIs** button to open the Scan Workspace.

Proceed to Select ROIs on page 39. If working in Scan Only mode, read more about this feature on the next page.

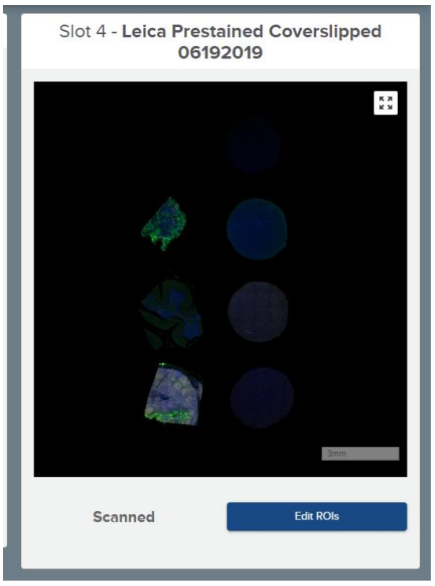


Figure 24: 20x scan and Edit ROIs button

Scan Only

Scan Only mode enables slide scanning and ROI selection without collection. ROIs selected on a Scan Only slide can be transferred to a Scan and Collect slide at another time using the ROI Transfer function (see ROI Transfer on page 56). This feature is useful when evaluating tissue staining conditions prior to the experimental run, or practicing/discussing ROI selection prior to ROI collection, for instance.

To scan a slide in Scan Only mode:

- Toggle the slider to **Scan Only** in the Scan Configuration window (Figure 25). Proceed through scan setup to generate a Scan Only record of the slide. When prompted, select **Edit ROIs** to open the **Scan Workspace.**

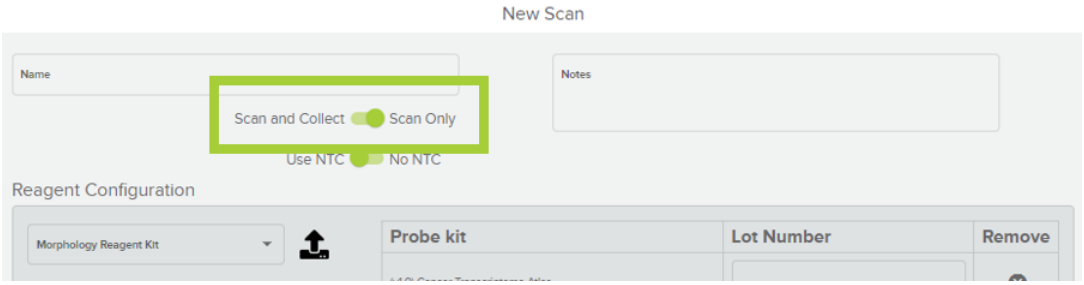


Figure 25: Scan Parameters set to Scan Only mode

To create a Scan Only slide from an existing scan:

- Copy an existing scan from the scan gallery by clicking **Copy** on its scan card (Figure 26). Select the attributes to copy to the new scan (ROIs, segments, and/or overlays). A Scan Only version of the scan is created on a card in the scan gallery. Click **Scan Workspace** on the card to create and edit ROIs.

Scans designated as Scan Only cannot be sent for ROI collection.

To move ROIs selected on a Scan Only scan to a Scan and Collect scan, use the instructions listed in **ROI Transfer** on page 55.

Figure 26: Scan card with Copy button

Select ROIs

The Scan Workspace (Figure 27) allows you to position regions of interest (ROIs) on your scan. If desired, you can also perform segmentation within the Scan Workspace or using an external program such as ImageJ.

You can work in the Scan Workspace in **Scan Only** mode or **Scan and Collect** mode. In Scan Only mode, scans can be evaluated and ROI selected, but will not be sent for collection (see Scan Only on page 38). In Scan and Collect mode, following ROI selection and approval, the GeoMx DSP instrument collects tags by exposing each segment of each ROI to UV light and aspirating material from the solution into a well of the collection plate.

With software v3.1, GeoMx DSP users can select and segment ROI through a third-party software, such as Visiopharm® Oncotopix® Discovery AI-driven precision pathology software. ROI and segments are passed back to the GeoMx DSP instrument using an OME-XML file. Please contact your NanoString Applications Scientist to participate in beta testing and obtain user documentation.

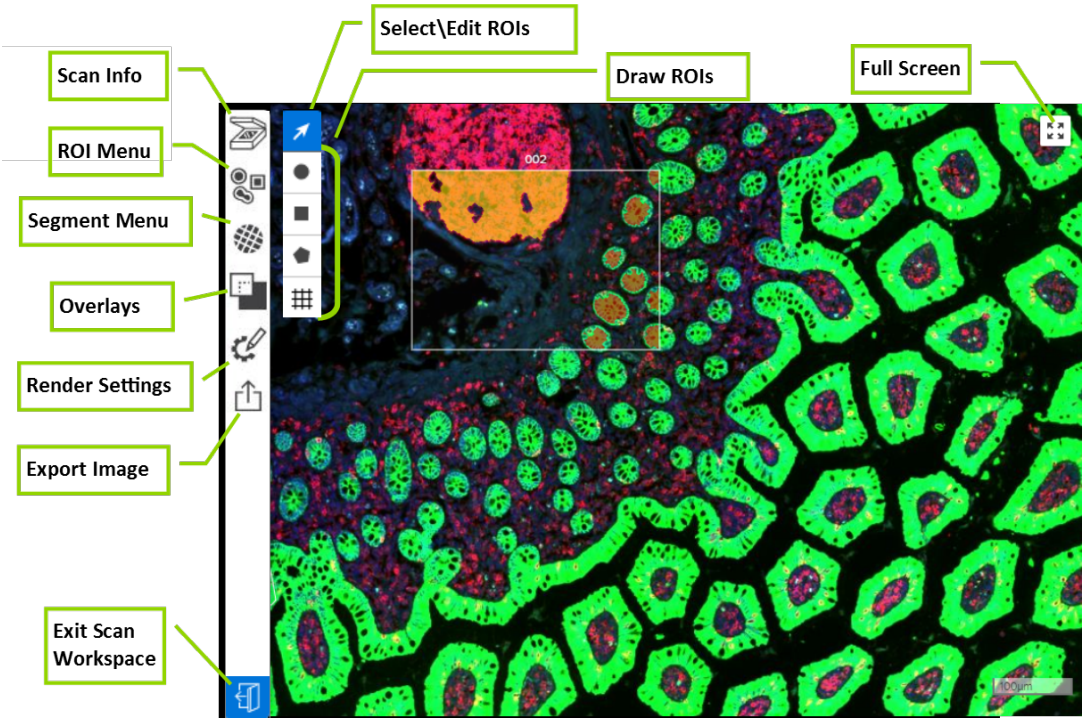


Figure 27: Scan workspace diagram

To select ROI while collaborating over videoconference, access the GeoMx DSP Control Center over remote Chrome connection from the computer which will run the videoconference. To view a slide presently in the instrument, click Data Collection, then New/Continue Run; or navigate to a Scan-Only mode scan in the Scan Gallery or Navigation window. Use screen-share to give your collaborators visibility to (and if desired, mouse control of) ROI selection.

Open the Scan Workspace

There are two ways to access the Scan Workspace:

1. During the DSP run, use the **Edit ROIs** button (Figure 28), which will become active underneath a slide's image after the 20x scan has completed.

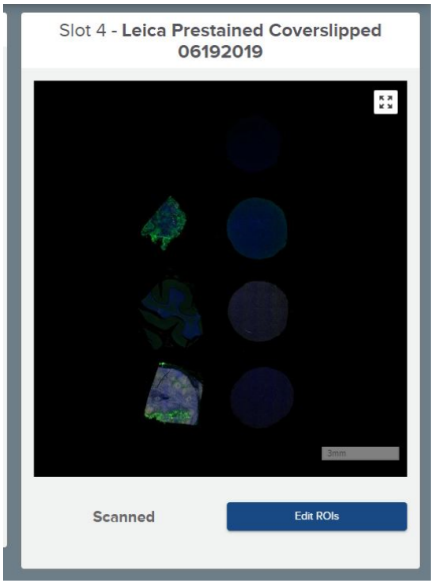


Figure 28: Control Center single slot view of Edit ROIs button activated after scanning

2. To access the Scan Workspace from outside of the DSP run workflow, select the Records button from the DSP Control Center. Use the Navigation window to browse to your scan and select it. On the scan card, select the Scan Workspace link (Figure 29).

Figure 29: Scan card with Scan Workspace link

Inspect the image

- Ensure that the scan quality is acceptable for designation of ROIs and segments. Do not proceed with collection from out of focus images, as it can cause instrument failure.
- Note that red blood cell autofluorescence is very common in FFPE tissues; avoid mistaking red blood cells (which do not contain nuclei) for nucleated cells.
- Open the **Scan Info** menu to review scan parameters, date created, and other details.
- Use the **Full Screen** control in the upper right corner of the scan image. **Zoom in** and **out** using the scroll wheel on your mouse or a pinch-zoom movement on your touch screen or touch pad. See the gray box above for keyboard commands for scrolling and zooming.

Keyboard Shortcuts

Zoom out	- (hyphen)
Super zoom out	_ (underscore)
Zoom in	=
Super zoom in	+
Pan image	(arrow keys)
Super pan image	Shift + (arrow keys)
Select pointer tool	1
Select circle tool	2
Select square tool	3
Select polygon tool	4

- Open **Render Settings** (Figure 30). Here, you can:

  • Change the colors used to represent the different channels on the scan.

  • Adjust the intensity of each channel, either with the slider bar or adjusting the values in the editable **Min** and **Max** boxes.

  • Use the **Undo**, **Redo**, or **Revert** buttons at the bottom of the Render Settings window, if needed.

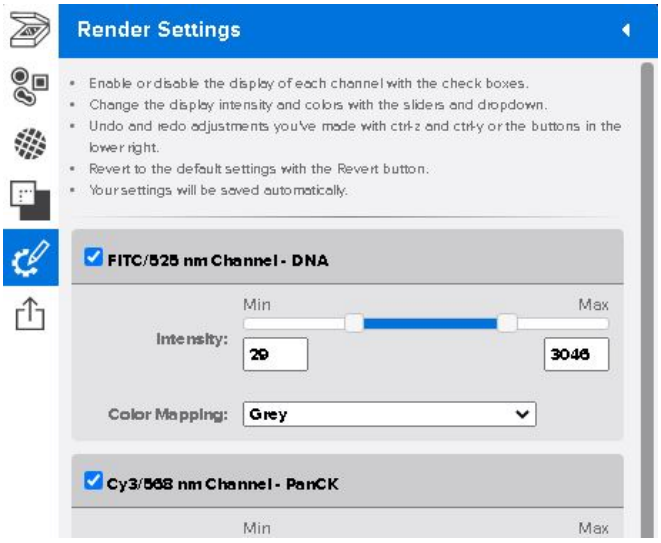


Figure 30: Scan Workspace Render Settings menu

Draw ROIs

Keep these considerations in mind when drawing ROIs:

- **For protein analysis, the area of an ROI** should generally be at least 2,000 μm^2 (approximately equal to a square with side length 45 μm or a circle with diameter 50 μm). ROIs that will be segmented should be larger. As a general guideline, the minimum number of cells per protein ROI (or, if segmenting, per segment) is 20. Open the ROIs List (see page 47) to check the ROI dimensions.
- **For RNA analysis, the area of an ROI** should generally be at least 30,000 μm^2 (approximately equal to a square with side length 170 μm or a circle with diameter 200 μm). ROIs that will be segmented should be larger. As a general guideline, the minimum number of cells per RNA ROI (or, if segmenting, per segment) is 100 for NGS readout and 200 for nCounter readout. Open the ROIs List (see page 47) to check the ROI dimensions.
- **Maximum** ROI dimensions are 660 μm x 785 μm. A maximum of 380 ROIs can be placed on a single scan.
- For **NGS assays,** the instrument designates the first well of each plate, and in addition the first well of any additional readout groups within a plate, as a no-template control (NTC). When planning your ROIs, segments, and plate usage, be sure to account for the NTC well(s). For more information about the NTC, refer to the GeoMx DSP NGS Readout User Manual (MAN-10153).
- For **custom standalone RNA–NGS assays** (*without* WTA or CTA), ensure that the ROI selection strategy includes a sufficient number of ROIs of sufficient size, or the resulting NGS library may not contain enough material for accurate library QC. A general guideline for a standalone custom RNA–NGS assay of 20 targets is a minimum collection area of 425,000 μm^2 (equal to 54 circular ROIs of 100 μm diameter), per pooled library. See GeoMx DSP NGS Readout User Manual (MAN-10153) for more information.

IMPORTANT: Do not establish ROIs and attempt to collect from areas of scan with poor focus, as this can cause instrument damage.

Standard Geometric Shapes

1. **Select a shape button (circle or rectangle)** from the Scan Workspace (Figure 31).
2. **Click and drag your mouse from the corner of the desired ROI area** to the desired size.
3. **Release the mouse click to complete the ROI.** This ROI will now have a number designation.
4. **To adjust,** click and drag the center point of the shape to move. Click and drag a white perimeter point to re-size.

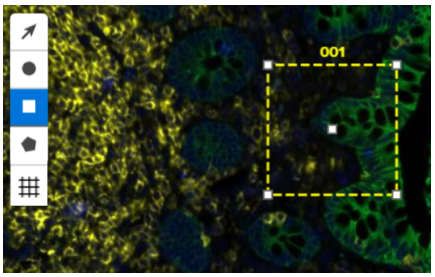


Figure 31: Scan Workspace square ROI button

Custom Polygon

1. Select the **polygon button** from the Scan Workspace (Figure 32).
2. **Single-click** where you would like one vertex of the polygon.
3. **Single-click to create more vertices** and build the ROI.
4. **Click back on the original vertex to complete the ROI.** This most recently applied ROI will now have a number designation.

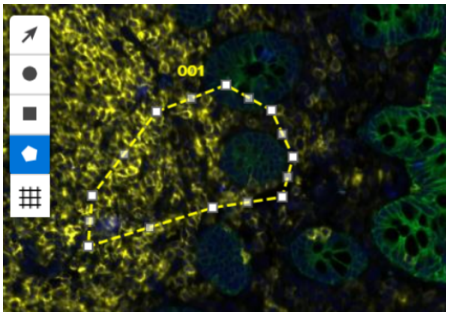


Figure 32: Scan Workspace polygon button

5. **Click and drag a vertex to adjust the polygon in shape and size.** If resizing causes unexpected changes in polygon shape and size, create a new polygon, then return to the polygon you wish to alter and it should resize properly.
6. Each side of a polygon has a center point; **click on a center point to convert it to a vertex**. Click on a vertex to convert it to a center point.

Keep in mind the minimum ROI size and cell number guidelines from the previous page. Placing a square ROI over or near your custom polygon ROI may help estimate its size. Open the ROIs List (see page 47) to check the dimensions of established ROIs.

Grid ROIs

1. **Select the Grid ROIs tool** in the Scan Workspace (Figure 33).
2. Select the **ROI shape** (circle or rectangle), **diameter,** and **column gap** (distance between each column of ROIs), **row gap** (distance between each row of ROIs), and **odd row indent** (to indent every other row, as in Figure 33).
3. **Click on the scan** in the location where you intend to place one corner of the grid and drag. The grid area will appear as a yellow dotted perimeter and the individual ROI previews will appear inside with white dotted line perimeters. The ROI count within the grid will appear in the lower right corner of the grid parameters field.

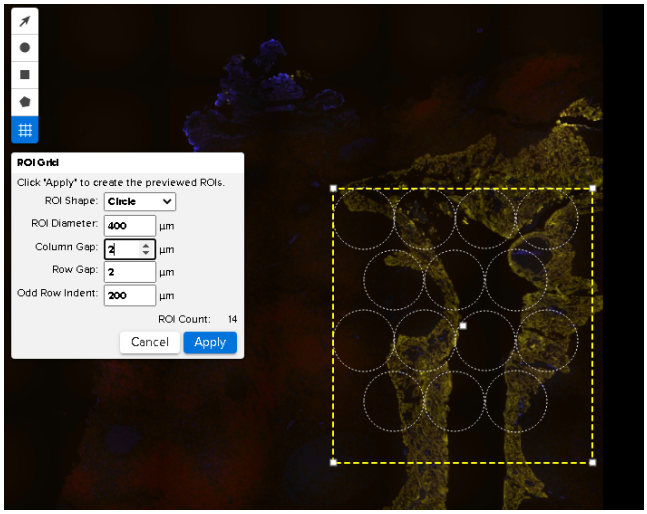


Figure 33: Grid ROIs tool

> Note that these are previews; the ROIs will not be applied to the scan until you complete the task by clicking the Apply button (next step).

4. When the preview appears as it should, click **Apply**. The ROIs will be applied to the scan.
5. Once you have placed the ROIs, they become separate ROIs that can be moved and altered individually.

> Keep in mind the minimum ROI size and cell number guidelines on page 43. A maximum of 380 ROIs can be placed on a single scan.

Move and Adjust ROIs

Select the **select/edit ROI** button (arrow icon) from the Scan Workspace (Figure 34) and click on the ROI you'd like to edit.

- Click and drag the center point of the shape to move it.
- Click and drag a white perimeter point to resize the shape.
- **Copy and Paste ROIs** by holding down the control button (Ctrl) on the keyboard and clicking and dragging the center point of an existing ROI. Release the click to place the new ROI(s).
- Select multiple ROIs by pressing Shift and then clicking the center of the desired ROIs.
- To delete the ROI(s), click the **Delete** or **Backspace** button on your keyboard. You can also click the red **X** in the ROI List (see next step). To delete all ROIs in the scan workspace, select an ROI in the ROI List, then press **Ctrl+A**, then press **Delete** or **Backspace**.

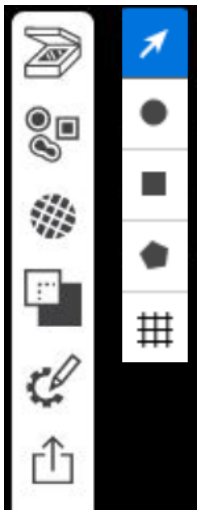


Figure 34: Scan Workspace select ROI button

Open the ROIs List (Figure 35) to:

- **Export all** ROIs as high-resolution tiled .tiff files for custom segmenting (see Using an External Program for Segmenting on page 54).
- **Import all** custom masks created in an external program.
- Toggle all segment/ROI masks on or off with **Show Segments.**
- Change the ROI numbering to any unique alpha-numeric choice. (Consider preserving the existing ROI number at the beginning of the name in order to maintain the order of the ROIs for collection). Do not use special characters like ' \ / * : < > | " '.? or begin/end with a space in the ROI or segment names.
- Manually change the dimensions or location of an ROI.
- View the nuclei count and area for each ROI and any individual segments. Please note the following:

  • Nuclei that exist on the edge of two segments may be counted in both segments.

  • The displayed ROI area is calculated in square micrometers whereas the segment area is based upon the number of pixels in the segment mask. As a result, a segment that encompasses an entire ROI may display a slightly different area than the ROI that contains it.

  • The segment area (μm^2) displayed in the ROIs tab of the Scan Workspace is an estimate to help guide segmentation. Final segment masks are computed once ROI selection is complete. Therefore, the actual segment area (μm^2) listed in the Lab Worksheet and Initial Dataset may differ from the value shown in the ROIs tab during ROI selection.

- Add a **Comment** to the ROI. ROI comments carry through to the Data Analysis Suite and are listed in the Manage Annotations template file. See GeoMx DSP Data Analysis User Manual (MAN-10154) for instructions to make ROI comments into tags or factors in your dataset.
- **Export** an individual ROI as a high-resolution tiled .tiff file for custom segmenting or **Import** a segment mask (see above).
- **Delete** an ROI. To delete all ROIs, select an ROI in the ROI List, press **Ctrl+A**, then press **Delete** or **Backspace**.
- View the current **Well Count** for this scan: the number of wells that the current selection of ROIs and segments will occupy in the collection plate. (NGS assays will also have an NTC well; see note on page 39).

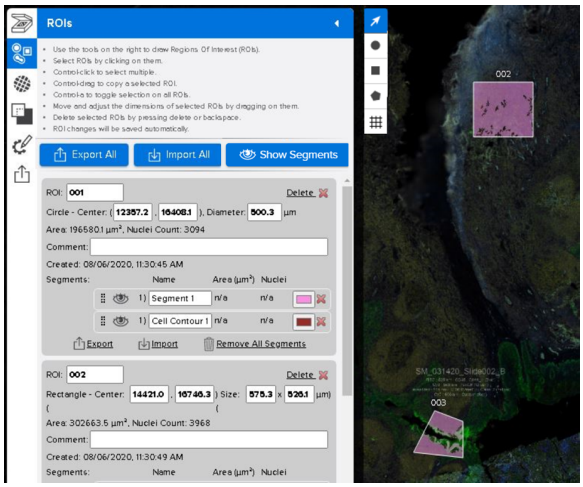


Figure 35: ROIs List

Segment ROIs (optional)

You can designate particular biological targets or combinations of biological targets as sub-regions, or segments, within your ROIs. Keep in mind the guidelines for minimum cell number per segment on page 43.

To auto-segment all ROIs in the scan, use the **Segments Panel** within the Scan Workspace. Alternatively, you can export an ROI image for processing in an external program, such as ImageJ. See Using an External Program for Segmenting on page 54 and **GeoMx DSP: Creating Geometric Segmentation Masks using ImageJ** guidelines in the NanoString University Document Library at https://university.nanostring.com.

> **IMPORTANT:** RNAscope can be used to help set the location of ROIs (i.e., place ROIs where a particular RNA is enriched). The signals from RNAscope, however, are not compatible with the segmentation algorithm on the GeoMx because RNAscope's signals are punctate. An advanced approach to combining RNAscope with segmentation could be designed by leveraging the Image Overlay feature (see Overlays, ROI Transfer, and Export Images on page 55).

Practice Exercise to Establish Thresholds for Segmenting ROIs

1. Select **+Add Segment Definition** one time to establish a single definition rule. One gray Segment Definition box will appear (Figure 36).
2. Channel designations determine whether each fluorescence channel (FITC, Cy3, Texas Red, Cy5) should be **included (+), excluded (–), or ignored (Ø)** as part of the segment rule's definition. **Choose one fluorescence channel and set it to +.** Leave the others set to Ø.
3. **Show Advanced Parameters** and set **Particle size** to **50** and all others to **0**.
4. Click the **Generate Segments** button to see the segments materialize on the scan.

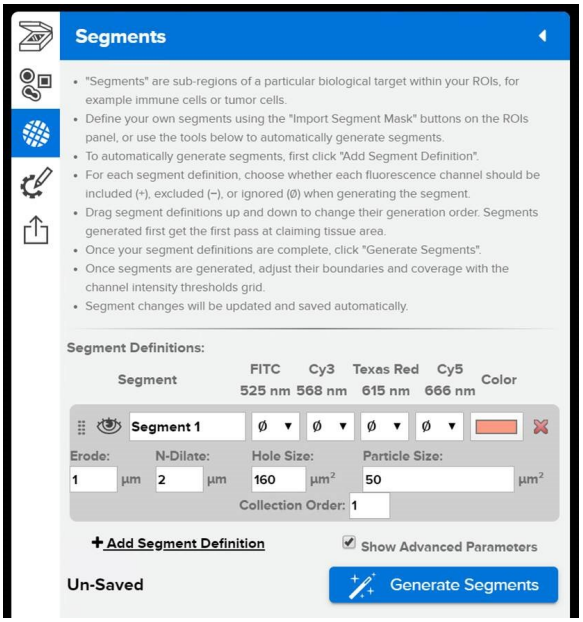


Figure 36: Scan Workspace Segmentation menu

5. Adjust the value in the **Channel Thresholds** grid (Figure 37), as needed, to change the sensitivity to the channel you've chosen to assess (the channel designation set to +). Consider starting with one ROI, adjusting the channel thresholds until the desired segment area is selected, then applying that value in **Override All**.
   - **Increasing the Channel Thresholds** number will increase the intensity required to be included in the segment and decrease the segment area.
   - **Decreasing the Channel Thresholds** number will increase the segment area.
   - Adjust until the desired area is selected.
   - Record the settings to refer to when you perform segmentation outside of this practice exercise.

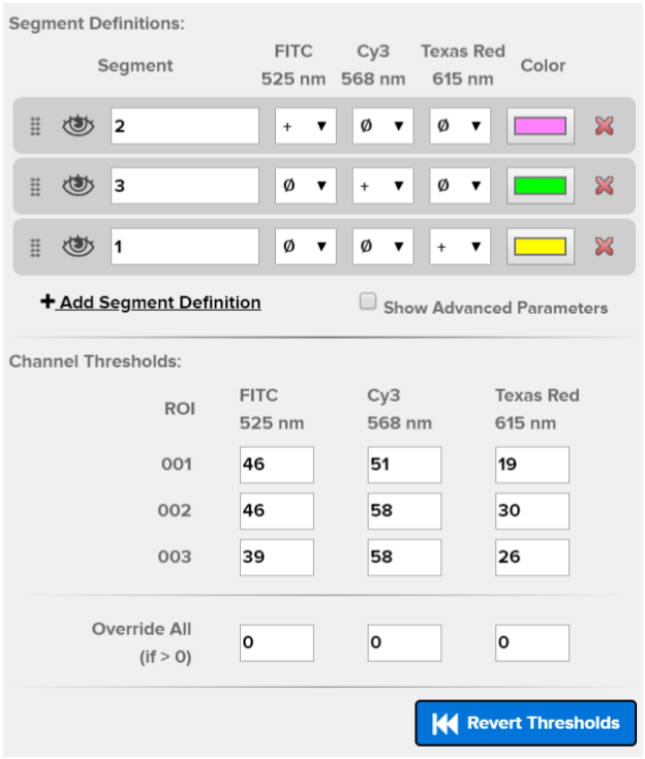


Figure 37: Segments with Channel Thresholds

6. Repeat steps 2-5, selecting a different channel designation in step 2.
7. Repeat until you have noted the desired setting for each of the channels in your analysis. Use these settings in Segmenting ROIs in the Scan Workspace on page 50. Delete the segment definition box used for this thresholding exercise.

Segmenting ROIs in the Scan Workspace

Keep in mind the guidelines for minimum cell number per segment on page 43.

1. Open the **Segments Panel** (Figure 38).
2. Select **+Add Segment Definition** one or more times to establish a definition for every segment you would like to create. These segments will be applied across all ROIs. Gray Segment Definition boxes will appear.
3. For each Segment Definition box (Figure 39), choose the **Segment Name** (to be used across all applicable ROIs). Names must have less than 175 characters, cannot contain special characters like ‘ \ / * : < > | “ ‘.? and cannot begin or end with a space. This segment name will transfer as a **tag** to the Data Analysis Suite. Alternatively, custom segment names can be added during data analysis by modifying the Manage Annotations template file. See the GeoMx DSP Data Analysis User Manual (MAN-10154) for more information.
4. **Channel designations:** For each segment definition box, choose whether each **fluorescence channel** (FITC, Cy3, Texas Red, Cy5) should be included **(+)**, excluded **(–)**, or ignored **(Ø)** as of the segment rule's definition (Figure 39).
   - Change the **segment color** used to illustrate the segment area on the scan, if desired.
   - **Delete a segment**, if desired.

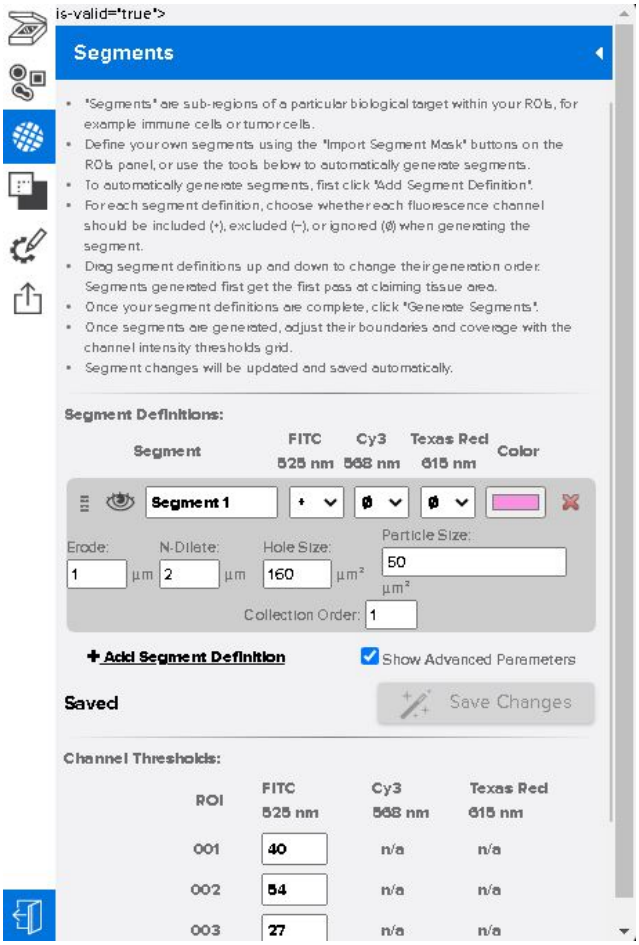


Figure 38: Scan Workspace segments panel

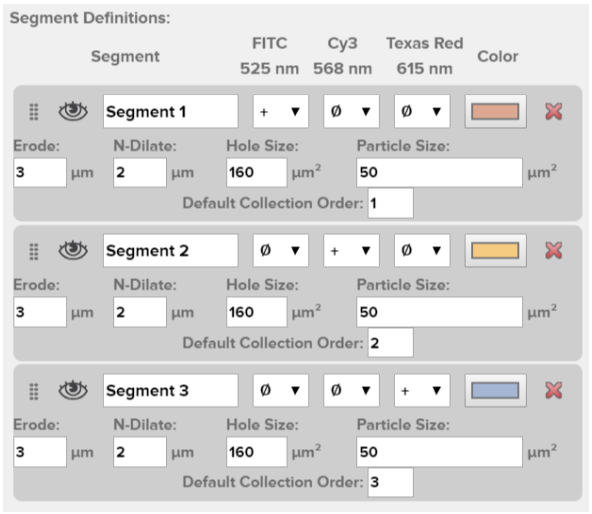


Figure 39: Segment definitions

5. **Segment generation order**: Use the grip bar to **drag and drop segment definition boxes** to establish the order in which you would like segments generated. This order will also dictate the order in which segments are collected, unless that order is changed in the Advanced Parameters (see below).
   - Generation order should be set in the order of increasing overlap. This means that **more specific segments should be generated first** (placed at the top of the list) and less specific segments last (placed at the bottom of the list).
   - The segment generated with the channel that is positive for DNA (usually FITC+), if used, should be generated last.
   - Areas included in an earlier segment will be removed/unavailable for later segments.
6. Click the **Generate Segments** button to see the segments materialize on the scan. If you make changes to the segment definitions, click the **Save Changes** button to ensure the changes are applied to the scan.
   - Select whether to **view/hide segments** on the scan by clicking on or off the **eye icon** (Figure 40). You can view/hide all segment/ROI masks using the **Show Segments** button on the ROI panel.

Figure 40: Visible segments and hidden segments clickable icon

7. Check the **Show Advanced Parameters** box to open **Advanced Parameters** (Figure 41). Here, you can adjust numerous parameters, including the order in which segments are generated and ultimately collected.

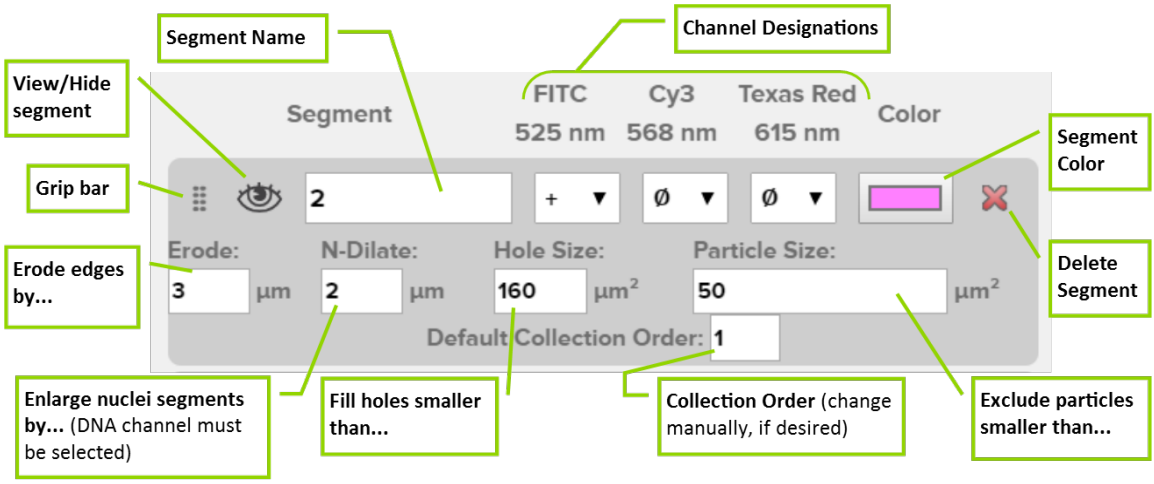
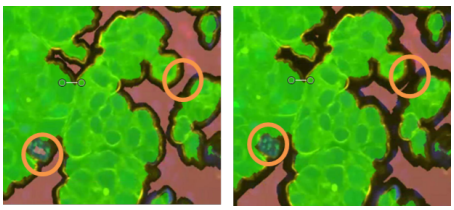
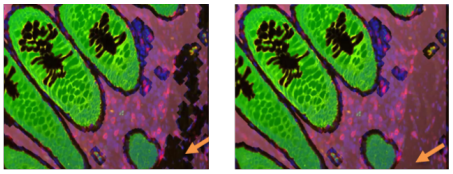


Figure 41: Segmentation advanced parameters

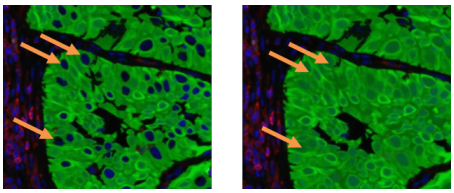
• **Erode:** The border width to be eroded from each segment; this effectively increases the boundary between segments. Increasing erode decreases segment size.



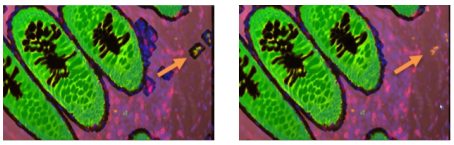
• **N-Dilate**: Adds segment area around detected nuclei. This setting is only applicable for nuclear-tagged segments.



• **Hole Size:** Holes smaller than this size in the detected segment area will be filled and included in the segment. Increasing hole size increases total segment area.



• **Particle Size:** Any small segment areas (particles) less than this size will be removed (despeckled). Increasing minimum particle size decreases total segment area.



Default Collection Order: The default order in which segments will be illuminated and collected, starting with 1. The default collection order will be the same as the generation order (see step 5 on the previous page), but it can then be modified independently of generation order.

8. After your first autosegmentation, a **Channel Thresholds** grid will appear below your segment definitions, allowing you adjust your segments manually for each ROI. Adjust the values in the **Channel Thresholds** grid, as needed, to change the sensitivity to each channel you've chosen to assess (the channel designation set to +) (Figure 42).

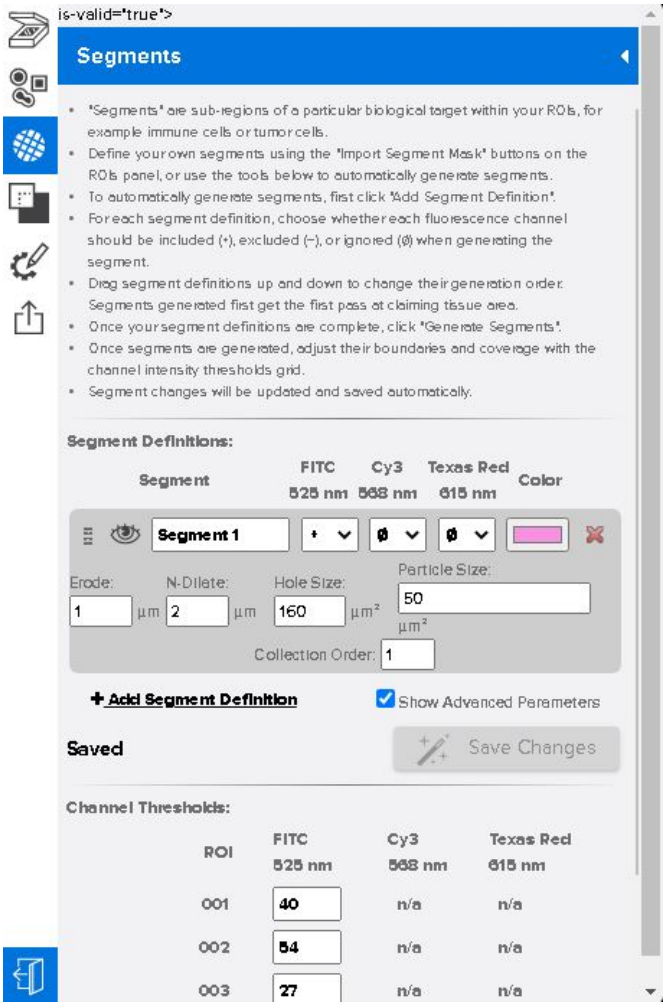


Figure 42: Segment definitions with channel thresholds

- **Increasing the Channel Thresholds** number will increase the intensity required to be included in the segment and **decrease the segment area.**
- **Decreasing the Channel Thresholds** number will **increase the segment area**.
- Note that changing a Channel Thresholds setting to 0 on an ROI will revert the channel threshold back to the default value.
- Adjust until the desired area is selected.
- Use the **eye** icon in each segment definition box to toggle all segments of that definition between visible and invisible.
- Use the **Channel Thresholds** grid to customize thresholds for each individual ROI or use the **Override All** row to customize thresholds to the same value for every ROI. For each channel, if there is a value greater than **0** in the **Override All** row, that value will be used for all ROIs for that channel.
- Use the **Revert Thresholds** button to revert to the default channel settings.
- Note that the collection order, well order, and name order of segments may differ from each other, as the system optimizes aspirate collection to reduce complexity.

IMPORTANT: If you made changes to segment definitions, click **Save Changes** before exiting the Scan Workspace. Otherwise, the changes to the segment definitions will not be applied to the scan.

Using an External Program for Segmenting

Export images to an external program (such as ImageJ) to create segments within an ROI.

1. From the ROIs List (Figure 43), select the **Export All** button to export all ROIs for custom segmenting. Alternatively, export one individual ROI using the **Export** button below the ROI.
2. The system will export files in multi-channel .tiff format. Save to a desired location.
3. Use the external program to create masks within the exported ROI. See the **GeoMx DSP: Creating Geometric Segmentation Masks using ImageJ** Guidelines at https://university.nanostring.com. NOTE: Segments cannot overlap.

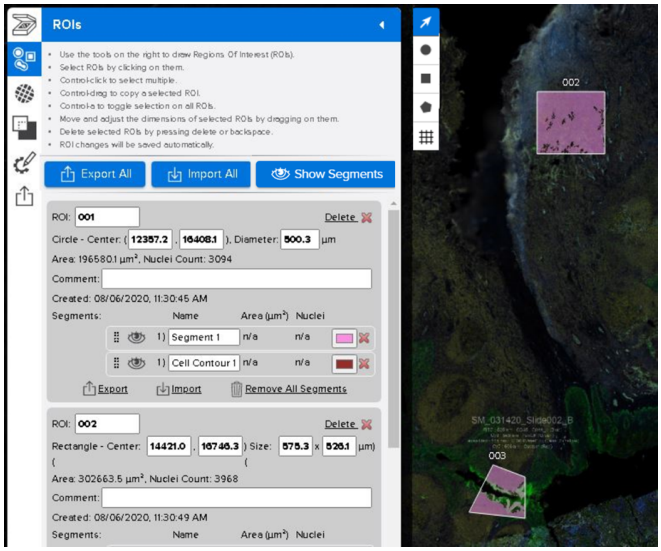


Figure 43: Scan Workspace ROIs List with Export Image link

4. Save the segment mask image to your computer as a .png or single layer .tiff file of the same resolution and dimensions as the original exported .tiff. Any pixel values greater than zero in your saved image will be treated as segment area. Zero-value pixels will be treated as empty space. Return to the Scan Workspace.

   If the filename does not have underscores, then the filename will be used as the segment name. If the filename has underscores, the system expects the filename in the following format:

   {roi-name}_{segment-name}_{collection-order}_{color}_{tag1}_{tag2}_{tag3}...

   All properties after {segment-name} are optional. {color} must be an HTML-style hexcode (like '10ffcc'). Imported segment names will be truncated at 100 characters, potentially resulting in non-unique names.

5. Select the **Import All** button from the ROIs List to import all custom segmented ROIs. Alternatively, select the **Import** button below each ROI of interest to individually import the applicable custom segment mask.
6. **Browse to the location of your saved image and select Open.** The segments will appear on the selected ROI.

Overlays, ROI Transfer, and Export Images

Overlays

You can overlay the current scan with imported images (including scan images exported from other GeoMx DSP instruments) to facilitate the selection of ROIs.

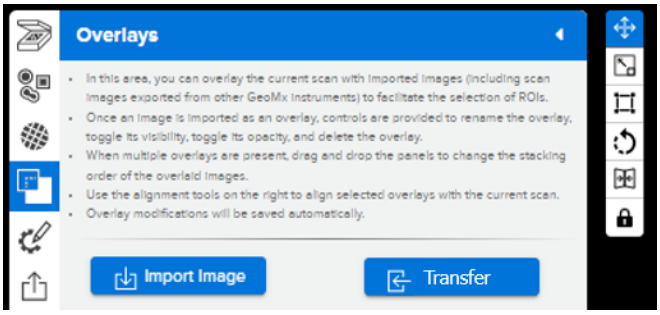


Figure 44: Overlays panel

1. Select the **Overlays** button in the Scan Workspace to open the Overlays Panel (Figure 44).
2. Click the **Import** button and **Browse** to your saved overlay image. You may import more than one overlay.

> Ensure your overlay does not exceed 32,767 pixels in width or height and is less than 3 GB in size. .png, .jpg, and .tiff file types are supported.

3. Each imported overlay image will appear on the scan and is represented by a gray box in the Overlays tab of the Scan Workspace panel. Here, you may rename the overlay, toggle its visibility, set its opacity, and delete the overlay. When multiple overlays are present, drag and drop the gray overlay boxes to change the stacking order of the overlaid images.
4. Click **Alignment** in the gray overlay box to reveal alignment tools on the scan (Figure 44). Alignment tools allow you to **Move**, **Scale**, **Resize**, **Rotate**, create a **Mirror Image**, and **Lock** the overlay. Use these parameters or the alignment tools that appear on the scan to adjust the alignment of the selected overlay on the current scan.
5. Click on the alignment tool you would like to use. There are three ways to align the overlay using each tool.
   - The three "handles" on corners of the overlay. These may not be accessible if zoomed in.
   - The alignment tool in the alignment box. Use the "adjust by" field to set the increment by which movements should be made.
   - The parameter fields in the alignment box can be incremented and edited manually.

Overlay modifications will be saved automatically.

ROI Transfer

ROI Transfer copies ROIs from an existing scan onto another scan. This feature is useful for:

- Transferring ROIs from one section to a serial section.
- Creating ROI templates for large slide cohorts (to keep numbers, types, and labels of ROIs consistent).
- Rescanning failed slides.

Only scans with status **Scanned** or **Annotated** can have ROIs transferred onto them.

1. Open the scan to which you will transfer ROIs in the Scan Workspace, and select the **Overlays** button to open the **Overlays Panel** (Figure 44).
2. Click **Transfer** to open the **Select Scan** window, and select the scan from which to copy ROIs. Click **OK**.
3. The ROI Transfer process begins and progress is shown on the screen. The time to transfer depends on the size of the scanned tissue and the number of ROI to be transferred.
4. An **overlay including ROIs** is displayed on the current scan. Adjust the overlay using the tools **Move**, **Scale**, **Resize**, **Rotate**, or **Mirror Image**, to position the ROIs as you wish.

- ROIs that are positioned outside the boundary of the destination scan will not transfer.
- ROIs that are resized and are now larger than the maximum or smaller than the minimum ROI size will not transfer.
- A rectangle ROIs that is rotated will transfer as a 4-sided polygon.
- A circle ROI that is stretched to an ellipse will transfer as a circle.

5. When you are satisfied with the incoming ROIs positions, click the **ROI button** of the Overlay Alignment tools menu (Figure 45).

Figure 45: ROI button of Overlay Alignment tools

6. Click **Transfer ROIs** (Figure 46). A confirmation message appears; click Cancel or OK.
   - Any ROIs transferred to this scan will overwrite existing ROIs on this scan.
   - If any ROIs are invalid, the system will list them and give the option to cancel (to realign the overlay and resolve the issue) or proceed to transfer the valid ROIs and exclude the invalid ROIs.
   - If transfer is still pending after 20 minutes, refresh the page.

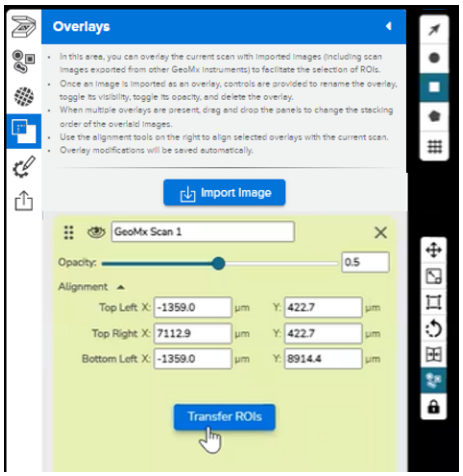


Figure 46: Transfer ROIs button

7. The transferred ROIs appear on the current scan. The overlay can be toggled on and off or deleted in the Overlays panel (and the ROIs will remain on the scan).
8. Open the **ROIs menu** (Figure 47) to see the transferred ROIs. You may adjust these or add additional ROIs.

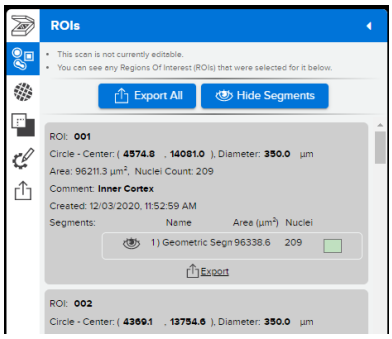


Figure 47: Scan Workspace ROIs Menu

9. Any segment definitions existing in the source ROIs will transfer to the new scan, overwriting existing segment definitions. **To apply the transferred segment definitions**, open the **Segments** menu and click **Generate Segments** (Figure 48). Segments can be adjusted or new ones created.

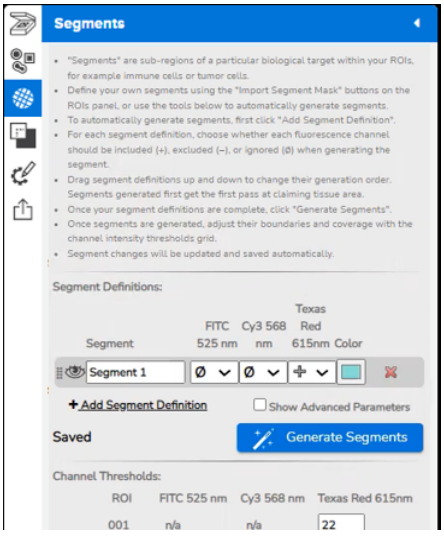


Figure 48: Generate Segments button

Export Images

IMPORTANT: Do not attempt to export images when collection is in progress. The instrument must be in an idle software state before exporting images.

1. Select the **Export** menu in the Scan Workspace.
2. The default view is **Rendered Scan Image** (Figure 49); choose this for a full-color publication-quality image.
   - Choose **Raw Channel Images** to export single-channel, high-resolution images as .tiff files. These may be several GB in size. If size exceeds ImageJ limit, try IrfanView graphic viewer.
   - Choose **ROI Report** for a zipped file with a separate image of every ROI and every segment within that ROI, as well as an HTML summary of those images.
   - Choose **OME-Tiff** to export as a .tiff file containing embedded OME-XML metadata.

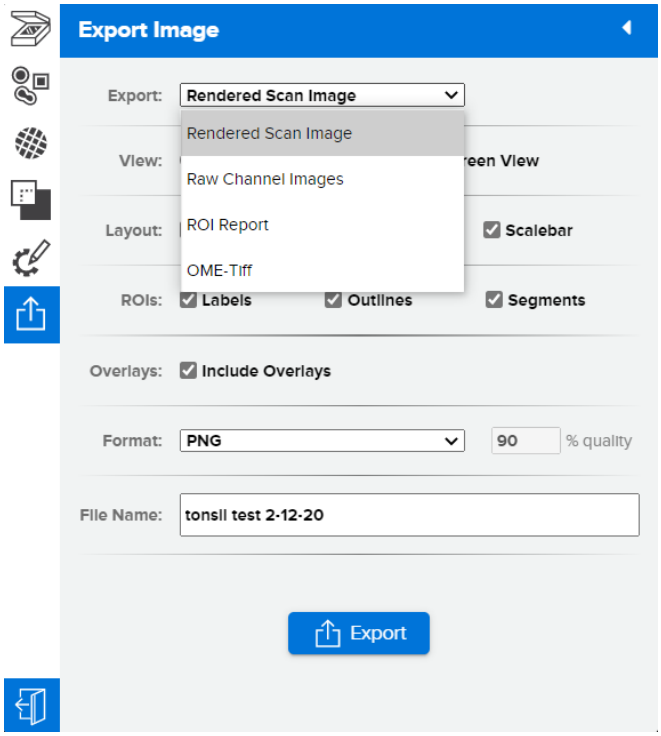


Figure 49: Export image menu - rendered scan

3. Use the **View**, **ROIs**, and **Overlays** fields to indicate what to export on the scan.
   - **Rendered Scan Image** allows you to choose **Full Scan**, which exports the entire scan image, or **On Screen View**, which allows you to manually zoom in and frame the export field as desired.
4. Choose the **Format** in which you'd like the image exported (.jpg, .png, .tiff).
   - The .jpg format allows you to adjust the file compression in the **% quality field**.
   - The full exported image will not be zoomed and will reflect the **Rendered Scan Image.**
5. Enter the **File Name** you'd like to use for the image.
6. Select **Export.**

Exit the Scan Workspace and Approve ROIs for Collection

> **IMPORTANT:** DO NOT attempt to begin collection if the scan is out of focus.

1. **Select the Exit button in the lower left** of the Scan Workspace toolbar (Figure 50) when ROI selection is complete.

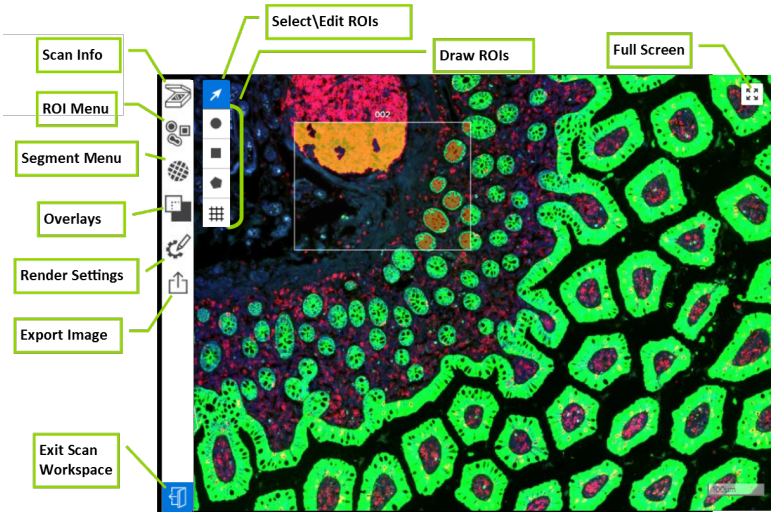


Figure 50: GeoMx DSP Scan Workspace with Exit button at lower left

2. If working in **Scan Only** mode, the Scan Workspace closes and you are returned to the scan gallery.
3. If working in **Scan and Collect** mode, a pop-up window asks you to **Approve ROIs** or **Save and Exit**. The **Well Count** estimate for your scan appears here (NGS assays must leave room for NTC wells; see note on page 43). **Approve the ROIs** to queue them for collection. **Save and Exit** to save the ROIs without starting collection. The Scan Workspace closes and you are returned to the slide view.
4. Repeat the steps **Select ROIs** and **Segment ROIs (optional)** for each slide. Use the **Edit ROIs** button to open the Scan Workspace for each scan image. Once ROIs have been saved and approved on a scan image, the button below it will change to **Modify** (Figure 51). ROIs can be modified up until collection from the slide has begun, then the **Modify** button will not be available.

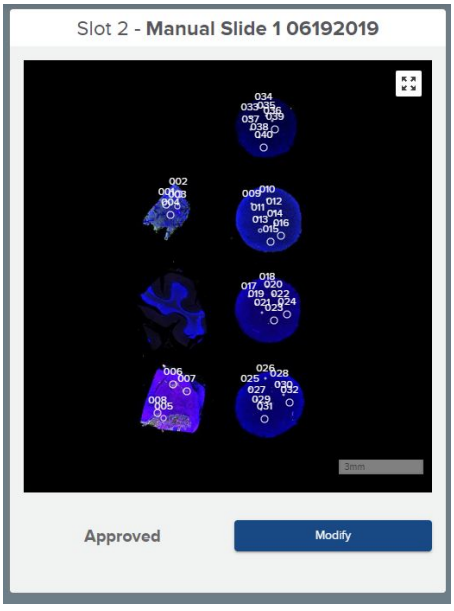


Figure 51: Scan status messages and buttons

If the collection plate becomes full, the GeoMx DSP instrument will pause and display prompts instructing to change the collection plate (Figure 52). If the instrument does not prompt to change the collection plate, log out (click on the username at top right, then Log Out) and log in again.

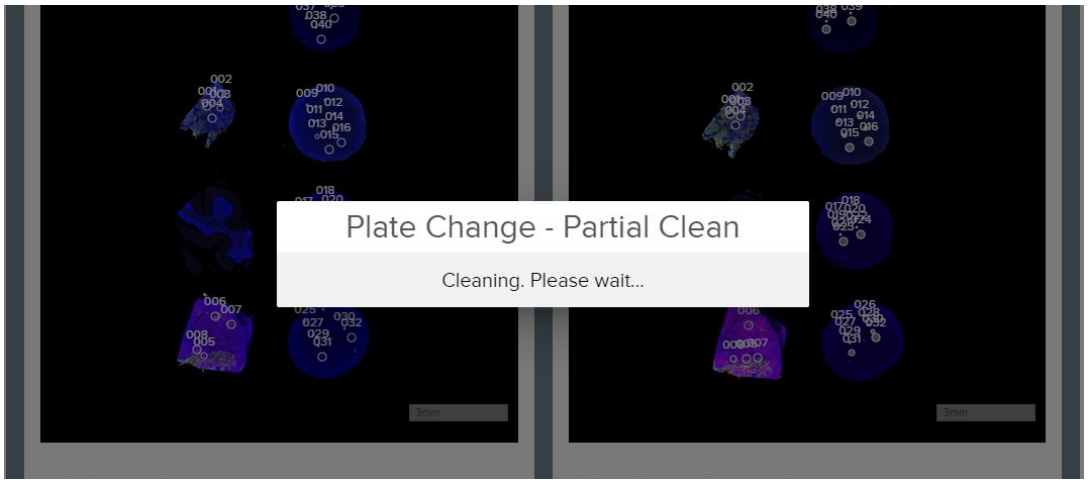


Figure 52: Plate change dialogue

NOTE: if placing a new plate for a collection with NGS readout, do not swap to a plate that was previously partially used for nCounter platform aspirates.

When all ROIs for each slide have been collected, the system will move on to the next step, Unload Plate and Slides on page 61.

Unload Plate and Slides

Once ROI collection is complete, the system will initiate an **Instrument Cleaning** process and end with the workflow marker at the top of the DSP Control Center at **Complete** (Figure 53). Each scan image will have the status **Collection Complete** displayed.

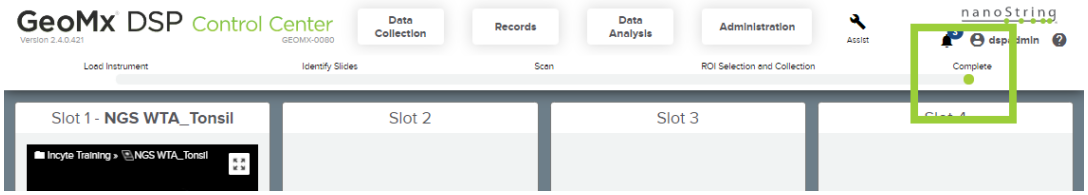


Figure 53: Workflow marker at Collection Complete

- Choose the **New Data Collection** button to begin a new collection. You will have the opportunity to exchange the slides and/or the collection plate.
- Choose the **Remove Slides and Microplate** button to remove these materials from the instrument, without initiating a new run.

Removing the collection plate

Follow the on-screen prompts to remove the collection plate from the instrument (Figure 54).

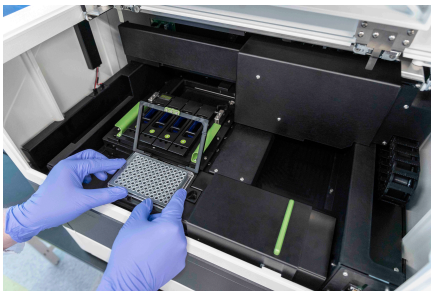


Figure 54: Unloading the collection plateon-on-on

After the collection plate is removed, it can be prepared for readout by NGS (see GeoMx DSP NGS Readout User Manual (MAN-10153) or GeoMx nCounter Readout User Manual (MAN-10089)).

If storing plate before processing for readout, seal plate with adhesive foil to prevent contamination. Store plate following these guidelines:

- If stored 24 hours or less: store at 4°C.
- If stored between 24 hours and 30 days: store at -20°C.
- If stored longer than 30 days: store at -80°C.

IMPORTANT: Deviating from the safe storage guidelines may result in reductions in data quality.

Removing the Slide Holder

1. Follow the on-screen prompts to remove the slide holder from the stage (Figure 55) and move it to a designated laboratory space. Do not close the instrument door yet, as you will return the clean slide holder to the instrument.
2. Using a pipette, remove and dispose of the buffer from each slide in the slide holder.
3. Open each slide slot clamp and unload each slide.
4. Store the slides:
   - **Protein slides:** Submerge in **1X TBS-T** at 4°C, protected from light, for up to 1 day. To store for >1 day, follow mounting procedure below.
   - **RNA slides:** Submerge in **2X SSC** at 4°C protected from light for up to **21 days**.

     Stability studies indicate that FFPE slides can be stored for 21 days in 2X SSC at 4°C without reducing the number of genes detected in tissue microarray and cell pellet array samples. Morphology marker signal remained functional for the duration of the study. SYTO 13 signal decreased, but remained functional. Nuclear or morphology marker stain can be repeated prior to scanning if deemed necessary.
5. Clean the slide holder and return it to the instrument. See Cleaning the Slide Holder on page 103.
6. Follow the prompts to close the instrument door.

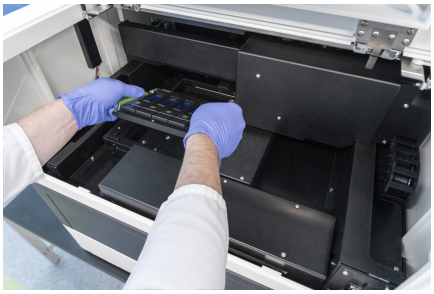


Figure 55: Unloading the slide holder

IMPORTANT: Store the slide holder in the instrument. Ensure it is in the instrument before closing the door.

Slide Mounting Procedure for Storage of Protein Slides (>1 day)

1. **Rinse slide** to be mounted with TBS-T or PBS-T. Touch the slide edge to a paper towel to remove excess liquid. Place slides on a flat surface.
2. Using a pipette tip (200 µL tip works well), **add one drop (~50 µL) of Fluoromount-G** to the slide; add more as necessary to ensure the slide does not dry out and there is adequate tissue coverage.
3. **Apply coverslip** (align one edge, then slowly lower from one side to the other) and remove excess mounting medium.
4. **Allow slide to dry** at room temperature in the dark overnight (such as bench drawer).
5. Store slide at 4°C, protected from light, for up to 3 months.

Finalize the Collection Plate

When you finalize the collection plate, you are designating the group of samples that will be processed together in the subsequent readout steps.

- Plates are **finalized by row**, not by well, even if some wells in the row are unused.
- Once you've finalized a set of rows in a readout group, **you cannot add more unfinalized rows** to that readout group. If you intend to add additional aspirates to a collection plate, do not finalize the readout group until all aspirates have been collected.
- You can later re-insert the plate and use the unused rows:
    - If the previous collection has been finalized, a different readout group must be used. Collection will begin in the next available row of the plate.
    - If the previous collection has not been finalized, the same readout group must be used. Collection will begin in the next available well of the plate, unless the new collection is with a different kit configuration, in which case it will begin in the next available row.
    - Some combinations of assays are not allowed in one plate (see Identify Plate on page 26) . If an incompatible combination is attempted, the instrument will prompt for a new plate.
- You can return to this window to access the readout files by clicking the plate icon and entering the plate number or searching by readout group name.

For NGS readout:

- Once finalized, plates can be moved between readout groups and multiple readout groups can be combined into a single readout group (with restrictions - see below) for processing all together using an Illumina NGS platform.
- The following combinations of plates are not allowed in one readout group: single analyte plates using Pro Code indices (IPA) **with** single analyte plates using Seq Code indices; single analyte plates **with** mixed analyte plates; or mixed analyte plates using Pro Code indices (IPA) **with** mixed analyte plates using Seq Code indices.
- You can change certain probe kit selections after ROI collection and re-download the readout package; use the new Pipeline configuration file to run the Pipeline, upload the new DCC files and create a new study. See page 71.

For nCounter readout:

- If needed, you can change the probe kit selection after ROI collection and re-download the CDF and worksheet, then create a new study in Data Analysis. Note that the probe kit will be applied to the entire row with which it is associated. See page 71.

1. To finalize a plate, click on the plate icon area at the lower right of the GeoMx DSP Control Center (Figure 56). If the plate is not currently in the instrument, enter its barcode.
   - After collection, the wells of the plate icon in the lower right of the Control Center should appear green for each collected well. Refresh (F5) to update the display. After Finalizing the plate, the entire row should appear green.

Figure 56: Plate icon in GeoMx DSP Control Center

2. The **Plate Information** or **Finalize Plate** window will appear:

For NGS readout:

The **Plate Information** window provides a table summarizing the status of each row of the collection plate (Figure 57).

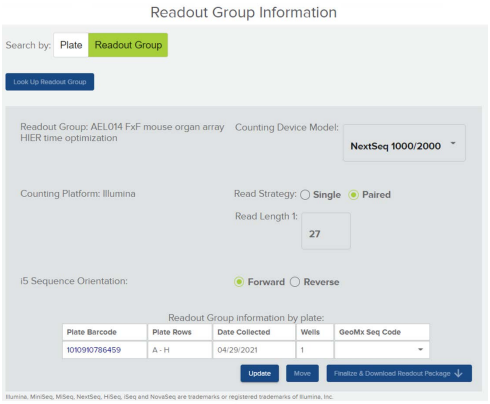


Figure 57: Plate Information & Readout Group window

1. Click **Readout Group** to see a list of available readout groups.

2. Select the appropriate Illumina platform from the **Counting Device Model** menu.

3. Choose **Single** or **Paired** Read.

4. Enter read length 27 into the **Read Length** field.

5. Choose the correct i5 Sequence Orientation (Table 4).

6. Establish which **GeoMx Seq Code or Pro Code** plate should be associated with the **Plate Barcode** in the **Readout Group Information** grid.

7. Click the **Update** button.

8. Select the **Finalize and Download Readout Package** button.

For nCounter readout:

The **Finalize Plate** window provides a table summarizing the status of each row of the collection plate (Figure 58).

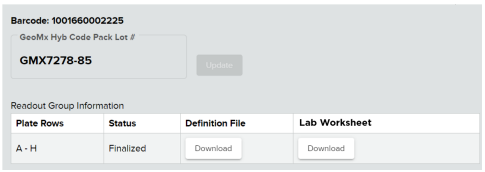


Figure 58: Finalize Plate window

1. Enter the **GeoMx Hyb Code Pack** lot number (Figure 59) to be used in downstream nCounter processing and click the **Update** button. If you do not know the lot number, you may skip this field and enter it when you upload nCounter counts.

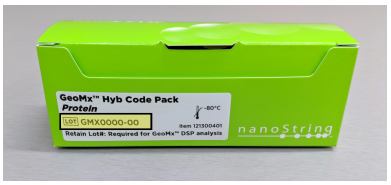


Figure 59: Hyb Code Pack lot number

2. The first time a Hyb Code Pack lot number is used, a pop-up window will appear. Click **New Lot** to continue finalizing the plate.

For NGS readout:
continued

9. When asked to confirm the **Readout Group** selection, click **Yes**.

10. Insert a USB drive into the instrument.

11. Click **OK** in the **Readout Package Successfully Created** window to save these files to the USB:
- **Lab Worksheet** contains information on the contents of each well of each plate, ROI coordinates, nuclei count, recommended sequencing depth, and more.
- **Configuration File** will be used by the GeoMx NGS Pipeline software to convert the Illumina FASTQ files into DCC files.
- **Seq Code or Pro Code UDI Indices** file is needed for your NGS Illumina platform run except when using the NextSeq 1000/2000.
- For NextSeq 1000/2000 users, a **samplesheet.csv** and **whitelist.txt** replace the Seq Code UDI Indices file.

12. NextSeq 1000/2000 users with their GeoMx DSP linked to their BaseSpace Sequence Hub workgroup: Click **Send to BaseSpace** to send the run details to the BaseSpace workgroup. A notification that the run has been created appears in the Notifications bell. Access through the BaseSpace Sequence Hub to start the run, check progress, and download results. See Administration Menu on page 77 and the GeoMx DSP NGS Readout User Manual (MAN-10053) for more details.

For nCounter readout:
continued

3. If you have just completed a run, the **Status** for each row of your collection plate that contained aspirate should read **Collected**. Once you finalize, the status for each row should read **Finalized**.

4. Select the **Finalize** button.

5. Insert a USB drive into the instrument.

6. Select the **Download** button in the **Definition File** column to initiate the download of the Cartridge Definition File (CDF) to the USB drive. The CDF is transferred to the nCounter MAX/FLEX/Pro platform (see the GeoMx DSP nCounter Readout User Manual (MAN-10089)). Do not edit the contents of the CDF.

7. Select the **Download** button in the **Lab Worksheet** column to initiate the download of a worksheet to use for reference during hybridization setup. This worksheet also contains information needed to set up readout on the nCounter SPRINT Profiler.

Table 4. i5 Index Read Direction - for Finalizing an NGS Plate

i5 Sequence Orientation	Illumina Platform
Forward	• HiSeq 2000/2500 • MiSeq • NovaSeq 6000 with v1.0 reagent kits • NextSeq 500/550 where local run manager software is used to set up sequencing run • NextSeq 1000/2000
Reverse	• HiSeq 3000/4000/X • NovaSeq 6000 with v1.5 reagent kits • NextSeq 500/550 without local run manager – sample sheet directly inputted into NextSeq 500/550 control software • NextSeq 1000/2000 set up with custom pipeline for BCL conversion (rare)

Uploading Counts

1. Click on **Data Collection** then **Upload Counts/Cal Files**. The **Upload Count Data and Cal Files** window opens (Figure 60).
2. Click **Choose File** and navigate to the **zipped** counts folder: DCC.zip or output.tar.gz for NGS readout, or RCC.zip for nCounter readout.

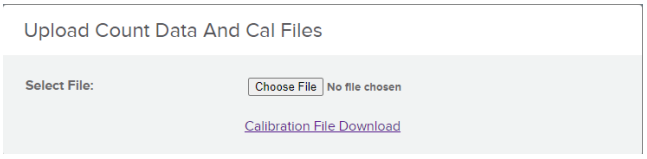


Figure 60: Upload Count Data And Cal Files window

3. If you encounter an error in uploading counts, check these points:

For NGS readout:

- Make sure there is not a subfolder within the DCC.zip folder.
- Make sure the DCC file names match the SampleID names in the SeqCode- or ProCodeIndices.csv of the readout package.
- Make sure that there is a DCC file for every sample in the readout group.

For nCounter readout:

- Make sure there is not a folder within the RCC.zip folder.
- Make sure the correct Hyb Code Lot number is associated with the experiment. Check by clicking on the plate icon.
- Make sure the correct CDF was used for the nCounter run.
- Make sure SampleID in the CDF matches SampleID and CartridgeID in the RCC files.
- nCounter data require a **Calibration file** for each new lot of Hyb Code. Download lot-specific Calibration files from http://www.nanostring.com/dspcalibfiles or directly from the instrument (**Calibration File Download**) (Figure 60).

Create Slides and Scans outside GeoMx DSP Workflow

Create a New Folder

You can create folders where you can organize slide records, scan cards, and analyses.

1. Select the **Records** button in the DSP Control Center (Figure 61).
2. This will open the **Navigation** window. Open a parent folder, if needed.
3. Click the **New Folder** button at the top of the Navigation window.
4. The system will prompt you for the following information:
   - Folder Name
   - Folder Description (optional)
   - Groups
5. Select **Save** to save the new folder.

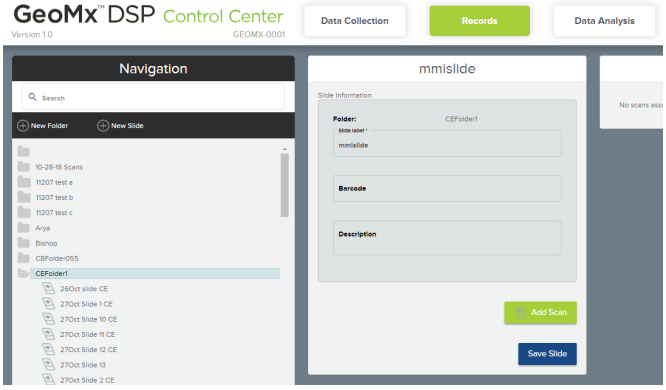


Figure 61: Records button and Navigation window

Create a New Slide Record

1. Select the **Records** button in the DSP Control Center.
2. This will open the **Navigation** window. Open or create a new parent folder.
3. Click the **New Slide** button at the top of the Navigation window.
4. The **Creating new slide record** window will appear to the right of the Navigation window. Fill in:
   - **Slide Label**: the unique identifier for this physical slide.
   - **Barcode** (optional)
   - **Description** (optional)
5. Select **Save New Slide**.

Associate the Slide Record with Scan Parameters

1. If the selected slide record does not yet have a set of scan parameters associated with it, the Scan Card reads, "No scans associated with this slide" (Figure 62). To associate scan parameters with this slide record, click the **Add Scan** button in the **Slide Record** window.

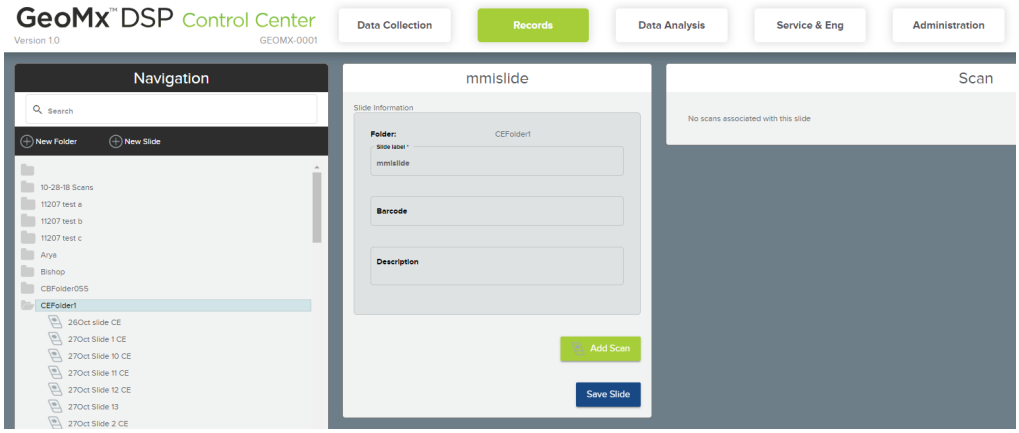


Figure 62: Slide record under Records in GeoMx DSP Control Center

2. The Scan Configuration window opens (Figure 63). Proceed to fill in scan parameters following the instructions in Create New Scan on page 30. If desired, save parameters in a scan template (see Scan Templates on page 34).

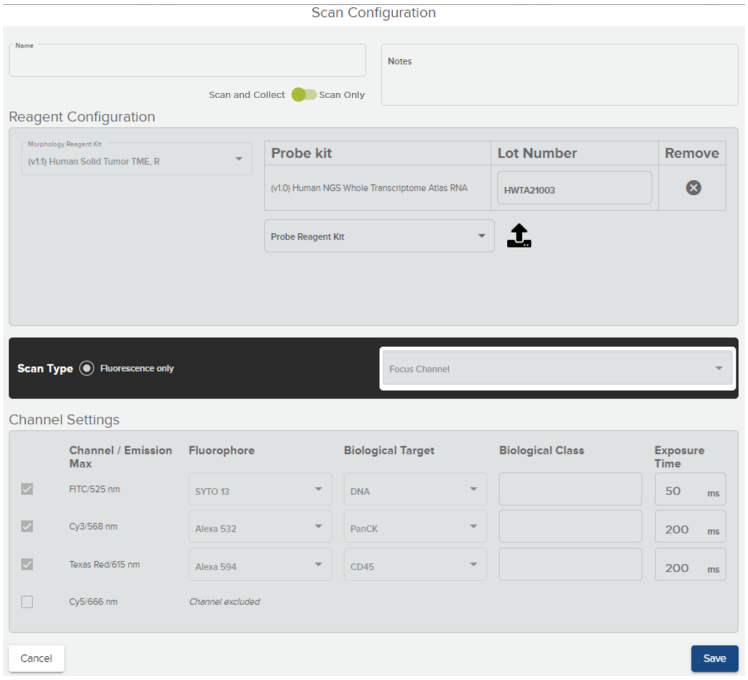


Figure 63: Scan Configuration window

Example Scan Parameters

For NanoString–provided Morphology Marker Kit reagents, the following settings are a recommended starting point for Scan Parameters (Figure 64 and Figure 65).

GeoMx Melanoma TME Morphology Kit, Human

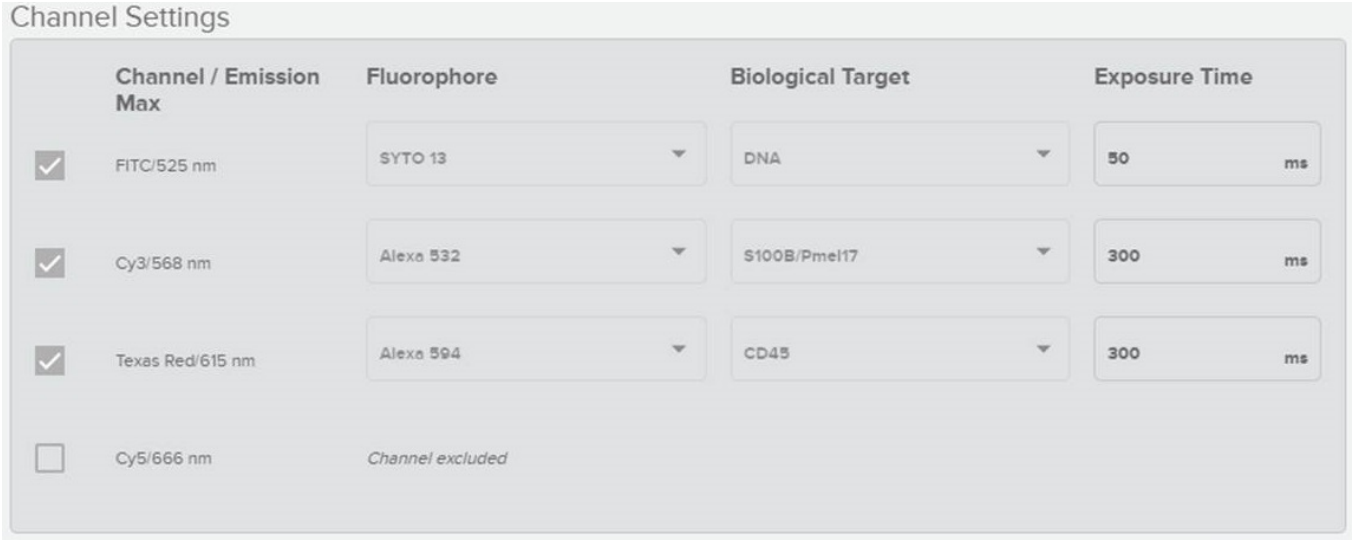


Figure 64: Example of GeoMx Melanoma TME Morphology scan parameters

GeoMx Solid Tumor TME Morphology Kit, Human

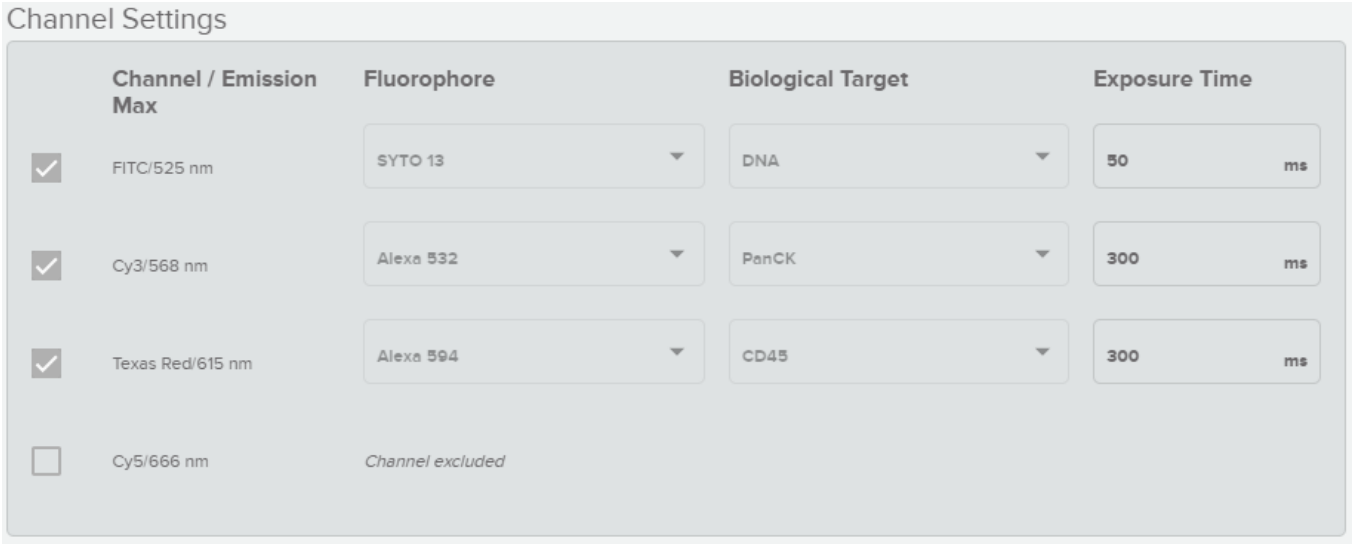


Figure 65: Example of GeoMx Solid Tumor TME Morphology scan parameters

Changing a Probe Kit Configuration File After Collection

Users may change the probe kit configuration (.pkc) files associated with a scan if a correction is needed, with some restrictions:

Change	Allowed?
Single analyte (RNA or Protein) to mixed (RNA + Protein)	No
NGS Readout: Mixed analyte (RNA + Protein) to single analyte (RNA or Protein)	Yes
Single analyte (RNA or Protein) to another analyte (e.g., RNA to Protein)	Yes, with restriction*
*Pro Code assay to/from Seq Code assay (i.e., Immuno-oncology Proteome Atlas to/from any other NGS Readout panel)	No

Slides must be in status **Readout Complete** to edit the associated .pkc(s). Therefore, users should proceed through the workflow, including the step of uploading counts (RCCs or DCCs) back to the GeoMx, yielding status **Readout Complete**. Then, modify the .pkc files and take the required steps to correct the readout, as follows.

1. From Scan Gallery, click on **Scan Parameters** on the scan card for a slide with status Readout Complete. The Scan Configuration window opens.
2. **Click the X** next to all Selected Probe Kits listed on the right to remove them.
3. **Add the correct probe kits** using the drop down menu on the left. First, select the Core Kit. Then, select any modules or custom kits. If you upload a new .pkc file, it must be **unzipped**.
4. Click **Save**.
5. To correct the readout:

For NGS readout:
Download a new Readout Package, since the contents of the configuration.ini file have changed with the modified probe kit configuration files. Rerun the GeoMx NGS Pipeline using the new configuration.ini file. If the resulting DCC counts files have the same names as the original DCC counts files, uploading them to the GeoMx DSP will overwrite the originals. Studies created from this scan will now show counts for the correct targets.

For nCounter readout:
Create a new data analysis study. Studies created from this scan will now show counts for the correct targets.

Slide and Scan Management

To manage slide records outside of the DSP run workflow, select the **Records** button in the DSP Control Center. This opens the **Navigation** window.

This navigation window (Figure 66) shows the hierarchy of all established slide records, scan cards, and Data Analysis studies. Use the search bar to filter records, if desired. In this window you can:

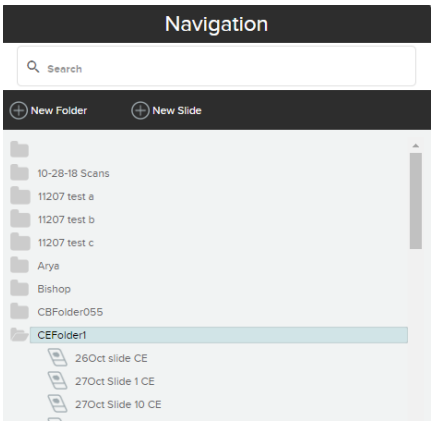


Figure 66: Navigation window under Records

- Select **New Slide** to create a new slide record.
- Select **New Folder** to create a new folder.
- Select a folder to view the slide records, scan cards, and studies within it.
    - Click on a folder icon to open/close that folder, but not select it.
    - Click on the name of a folder to both open/close it and select it.
- Select a slide record or scan card to view.
- Select a study to open.
- Move files/slides/studies by dragging and dropping.
- Move multiple files/slides/studies using Ctrl-click.
- Delete scans or studies by right-clicking on them, then selecting **Delete**. See Deleting Scans on page 76.

Scan Cards

A slide record that has scan parameters associated with it will automatically generate a Scan Card. When the scan is complete, the scan card will include the scan image and a summary of the scan's status and info.

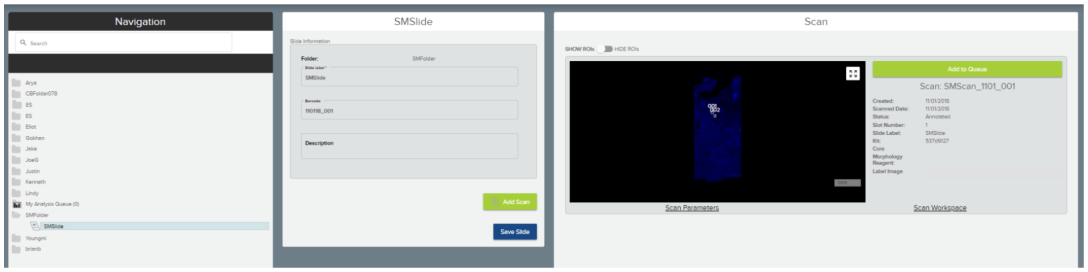


Figure 67: Navigation window, slide record, and scan card

Select a single slide from the list in the **Navigation** window. The Slide Record and any Scan Cards for this slide will appear (Figure 67). You may select:

- **Hide or show ROIs** using the slider.
- **Copy** to copy the scan to a Scan-Only version.
- **Delete** to move the scan to the Trash folder.
- **Add to Queue** to send to Data Analysis queue (enabled if scan's status is Readout Complete).
- **Scan Parameters** to view the Scan Configuration window.
- **Scan Workspace** to open the Scan Workspace window.

The listed **Status** for the scan will inform on what stage it is in according to the GeoMx system:

- **Defined:** scan is set up.
- **Scanned:** scanning complete.
- **Annotated**: ROIs selected.
- **Aborted:** scanning or collection was aborted.
- **Collection Complete**: collection complete, rows not finalized.
- **Awaiting Readout:** rows finalized.
- **Calibration Needed**: the system needs a **zipped calibration file** for applicable Hyb Code lot to be uploaded - find at https://nanostring.com/products/geomx- digital- spatial- profiler/geomx- dsp- calibration-files/. See Uploading Counts on page 67.
- **Readout Complete**: counts uploaded, ready for Data Analysis.

Managing Scan Cards

Select a folder from the Navigation window. Click the grid button or bars button in the top right to see alternative **gallery views** of the scan cards in that folder (Figure 68).

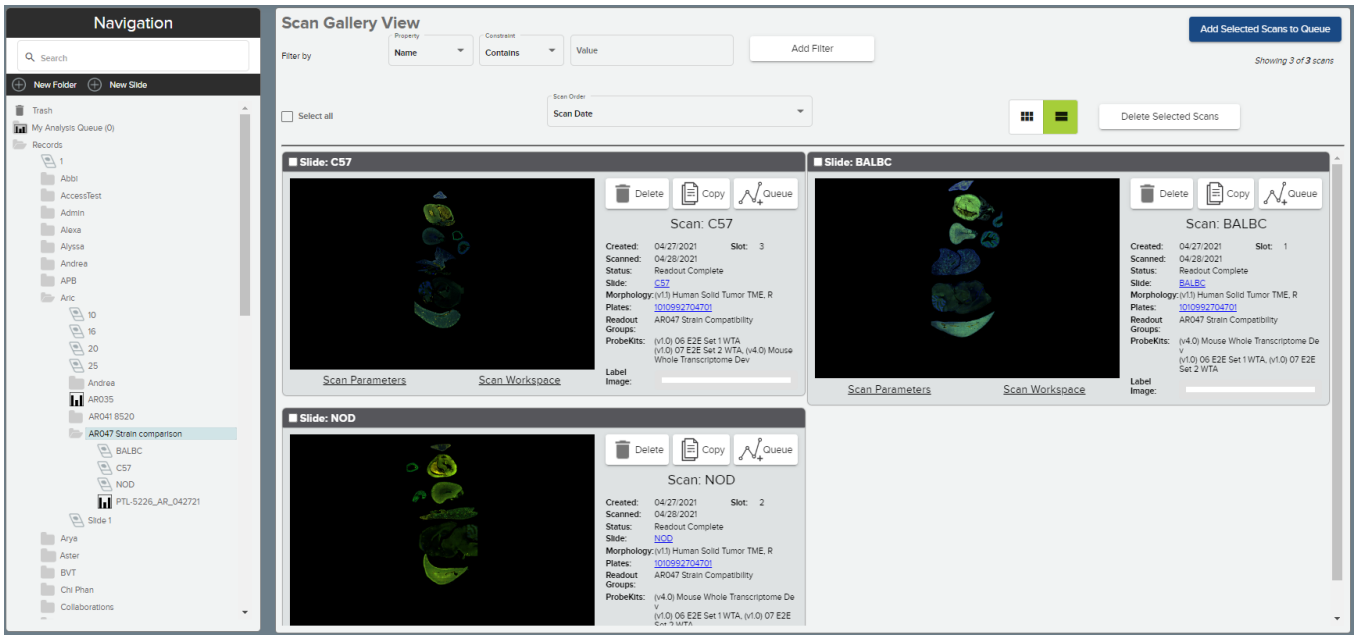


Figure 68: Scan gallery view

Filter by

Use slide and scan metadata to filter scans. Select search parameters from the dropdowns in the upper left of the window (Figure 68).

- **Property**: Name
- **Constraint**: Contains
- **Value**: customizable field
- **Scan Order**: Scan Date, Scan Name, Slide Name

Select **Add Filter** to apply a filter rule. Applied filter rules will appear as gray boxes under the filter field. Use the **X** on each box to remove that filter, if desired.

Select **Clear Filters** to clear applied filter rules.

Select all, Hide ROIs, & Scan Order

- **Select all** scans using the check box. Alternatively, you may select one scan at a time using the check box in each scan's upper left corner.
- The **Scan Order** default is by scan date (from most to least recent); select another option from the dropdown (Scan Name or Slide Name), if desired.

Once all desired scans have been selected, click the **Add to Queue** button to send this scan's counts to the Data Analysis queue. See the GeoMx DSP Data Analysis User Manual (MAN-10154).

Editing or Deleting a Folder

Highlight a folder in the Navigation window and right-click.

- Select **New Folder** to create a new folder under the highlighted folder. See Create a New Folder on page 68.
- Select **Edit** to edit the highlighted folder.
- Select **New Slide** to create a new slide record under the highlighted folder. See Create a New Slide Record on page 68.
- Select **Delete** to delete the highlighted folder. Only empty folders may be deleted.

Deleting Scans

Scans must be in one of the following states to be deleted: Readout Complete, Scan Only, Error, or Aborted.

Delete scans and studies from the system by right-clicking on the item's name in the navigation window and selecting **Delete** (Figure 69).

The software asks to **confirm** the deletion in a pop-up window.

The deleted item is then moved to the **Trash** folder, listed as the first item in the navigation pane.

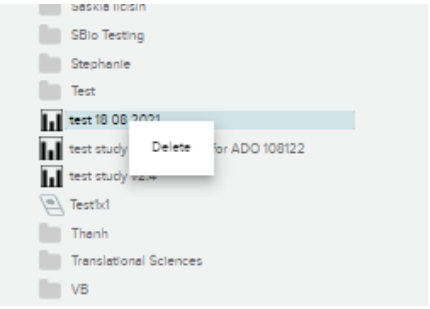


Figure 69: Delete an item.

Click on the Trash folder to view the studies and scans in the Trash (Figure 70).

From the Trash folder, deleted items may be **restored** to Records by selecting the item and then clicking **Restore**.

Administrative-level users have the additional option to **permanently delete** an item by selecting the item and clicking **Delete**. This action removes the item from the instrument and the archive. **It cannot be restored.** Disable the **Delete only items successfully transferred to AtoMx SIP selection** at the top right of the Trash window to enable permanent deletion. (AtoMx SIP connection will be enabled in an upcoming software release).

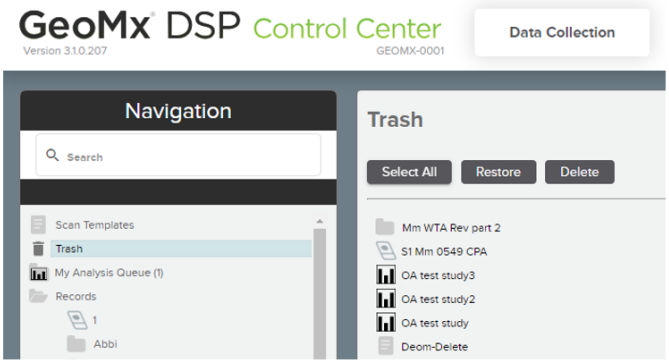


Figure 70: Trash folder with Restore and Delete buttons outlined.

NOTE: When a top-level item (e.g. folder) is moved to the Trash and deleted, all sub-level items will be deleted. Sub-items cannot be selected individually for deletion from the Trash. To delete an individual item, restore the top-level item to the Records folder, then select the individual item for deletion.

Administration Menu

The **Administration** button in the DSP Control Center is active for both administrative and general users. **General users** only have access to AtoMx SIP configuration, download logs, error reset, and system settings (to change system time and shut down the instrument).

In addition to the above functions, **administrative users** can access user and group accounts, manage email notifications, manage files and kits in use, configure a data analysis auxiliary server, configure a connection to Illumina® BaseSpace, manage network and SSL settings, update the software, and access other system details (Figure 71).

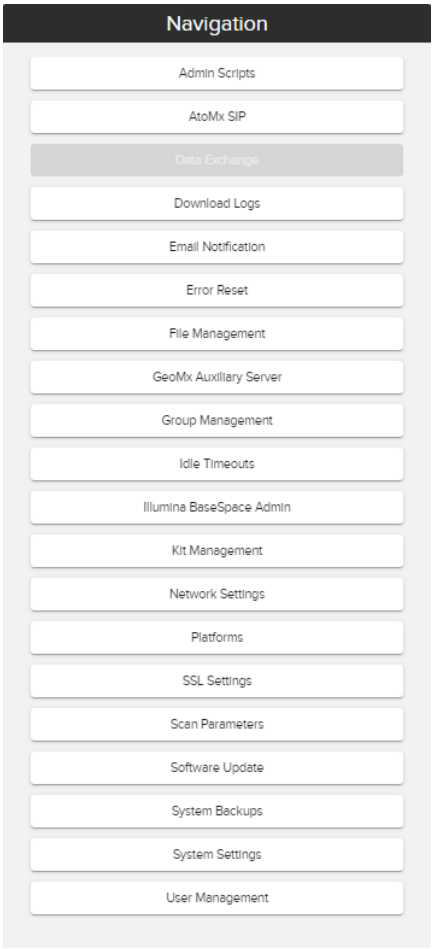


Figure 71 : GeoMx DSP Administration menu. Data Exchange is not available over remote connection.

Admin Scripts

The Admin Scripts tab allows you to run individual scripts for specific purposes. **Run only under instruction of NanoString personnel.**

- **ADMIN_SetSystemForWastePumpFlush** is designed to set the DSP instrument into the right valve states to flush the waste pump with a syringe and filter (this requires a kit from NanoString).
- **ADMIN_RestoreSystemAfterWastePumpFlush** restores the DSP system into the right valve states and runs a script to make sure the waste pump has recovered from a previous procedure.
- **ADMIN_SystemPrimeFromBottle** is for recovering from a fluidic error on the needle if it has pulled air into the system.
- **ControlDoor** lets you unlock/lock door. A prompt will appear to ask if you want to lock the door. Type **True** or **False**.
- **RestartInstrumentService** restarts the instrument services.

AtoMx Spatial Informatics Platform (SIP)

GeoMx DSP connection to AtoMx SIP will be enabled in an upcoming software release.

Data Exchange

Data Exchange allows you to move GeoMx DSP scans and studies to and from any GeoMx DSP instrument. This function is only available when logged in locally to the GeoMx system, not over remote Chrome connection. External drives must be NTFS or exFAT format (NTFS recommended) and USB 3.0 is recommended over USB 2.0. Seagate Expansion Portable Hard Drives work well. **Do not use a drive greater than 10 TB.**

Exporting GeoMx scans and studies

1. Plug the external drive into the GeoMx instrument's USB port. Log into the GeoMx system as an administrative user.
2. Click on **Data Exchange** from the Administration menu. Within the Data Exchange window, navigate to the scan or study to export (Figure 72). Use **Ctrl+click** to select multiple files. It is not possible to select entire folders.
3. Once the selection(s) have been highlighted, select **Export.**

   > Any exported study must have the scan data to accompany it. The GeoMx system will automatically include any scan data with an exported study.

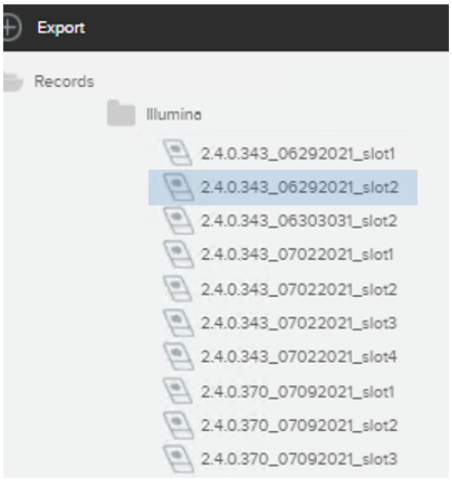


Figure 72: Navigation window for Data Exchange study selection

4. View available external drives (NTFS or exFAT format). Consider available space on any drives present and the estimated size of the export (Figure 73). Select a drive to which to export and click **Confirm.**
5. The Notification Bell in the Control Center will inform when export is complete. Allow the export to fully complete before starting a new export.

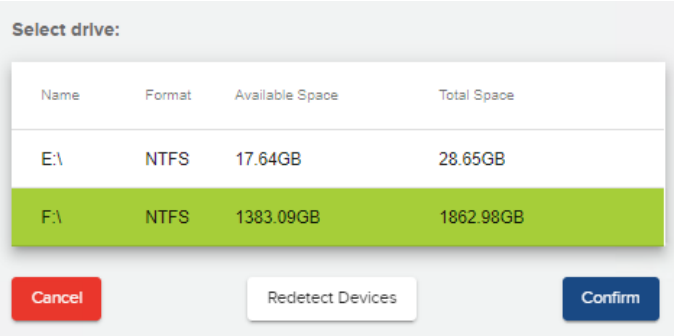


Figure 73: Selecting external drive

Importing GeoMx scans and studies

1. Plug the external drive into the GeoMx instrument's USB port. Log into the GeoMx system as an administrative user.
2. Click on **Data Exchange** from the Administration menu. Within the Data Exchange window, navigate to the folder into which you would like import.
3. Once the desired folder has been highlighted, click **Import**.
4. View available external drives. Select a drive. Click **Confirm**.
5. The system will display all GeoMx files found on the selected drive. Highlight the desired file and click **Select**, then **Confirm** (Figure 74).

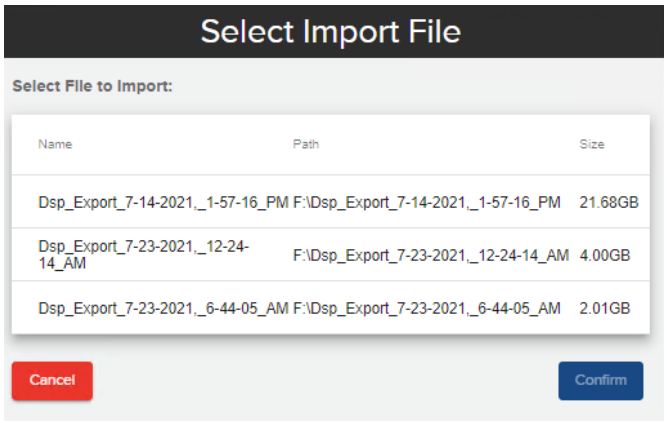


Figure 74: Select Import File window

6. The Notification Bell in the Control Center will indicate when import is complete.
7. If an imported folder does not display both slides and studies, right click on the folder, select Edit, and click Save. The slides will then display.

NOTE: If data already exists on the instrument in one folder location, and you try and import again to a new folder location, the new location will not be populated with the data.

Download Logs

- Select the date range of interest (Figure 75).
- Select whether to include all images, no images, or only support images from the dropdown.
- If logged into the instrument directly, insert a USB drive. **Do not use a drive greater than 10 TB.** Log files will be exported as a set with filenames that indicate which set they belong to. Logs will be exported in an unencrypted/non-password protected manner to the root level of the attached USB drive.
- If logged into the system remotely, log files will be exported to the remote user's Downloads folder (or other defined destination).
- Navigating away from the GeoMx window while downloading a large file may interrupt the download. For best results, do not navigate away until the process is complete.

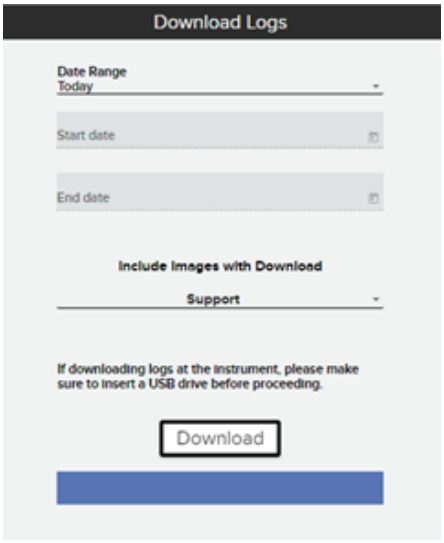


Figure 75: Download Logs tab under Administration

Email Notification

If desired, check the box to **Enable Email Notification** (Figure 76).

In **Notification Email Address**, enter the email address to receive notifications. To notify a group of GeoMx DSP users, please work with your IT department to configure an email alias or distribution list. Fill in the **SMTP** fields with help from your IT department.

In **From Email Display Name**, enter the name to display in the From field of the notifications. In **From Email Address**, enter an email account set up by your IT department on the server host domain for the purpose of GeoMx DSP notifications.

Check the boxes to **Use TLS** (transport layer security protocol for internet communications) and/or **Use HTML For Message Body** (optional), and select the events for which you would like to be notified. Click **Save**.

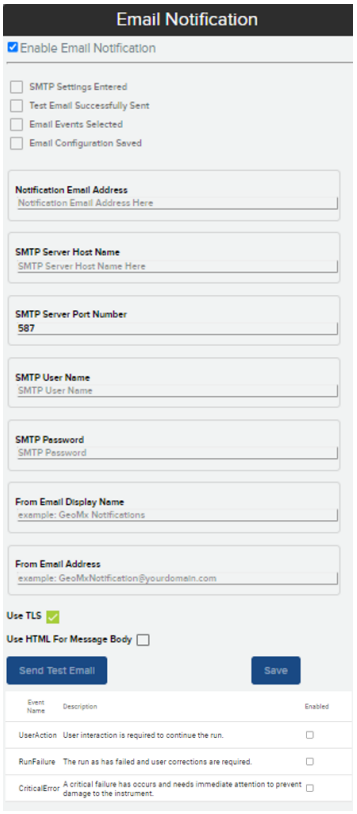


Figure 76: Email notification window

Error Reset

> **IMPORTANT:** It is recommended that you always contact Support@nanostring.com before attempting Error Reset.

Clicking on the **Reset Error Level** button will result in the following warning:

Warning: Resetting data collection errors without correcting underlying causes or first contacting NanoString Support may result in future sample loss or instrument damage.

If you choose to proceed, the error reset signal will be sent to the system and the result of this action (Success or Failure) will be displayed.

Following Error Reset, wait at least two minutes before attempting to change reagents or start a new instrument run.

File Management

1. Select File Management to set archive settings (Figure 77).

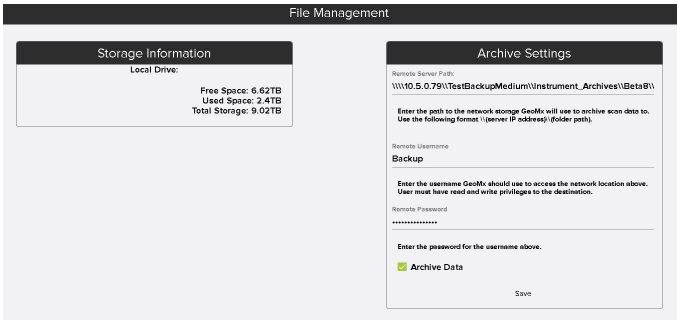


Figure 77: File Management tab under Administration

- **Remote server path:** Enter the ***network path*** in UNC format for the remote location where scan data will be uploaded. The remote server path must contain four backslashes, the IP address and folder path, in this format:

  \\\\server IP address\\folder path

  Since archiving from the GeoMx DSP system can occur at any time, the remote location must be available at all times.

  If multiple GeoMx DSP instruments are configured to archive to the same server, each instrument must write to its own archive folder.

- **Username:** enter the username for a user with read and write privileges for the remote server path entered. If including the domain, use this format: user@domain.
- **Password:** enter the correct password for the username. Passwords may not include $! /

2. Check the **Archive Data** box at the bottom of the form.

3. Click **Save.** A confirmation or error message will appear.
4. After first turning on archiving, the GeoMx system will create a file titled *mount-verification.txt* in the archive location. Check the archive destination folder to confirm this file was created successfully. This indicates that archiving is working properly. If the expected file is not present, contact Support@nanostring.com to troubleshoot.

A list of dates and location of system backups can be viewed by clicking **System Backups** under **Administration**.

In the event that you received an error when setting up the archive, check the following specifications and tips:

- Ensure that your institute network is set up for SMB3 file sharing.
- Enter the Remote Server path as \\\\123.45.6.78\\FolderName using the server IP address and your designated folder.
- Try the username in different formats, such as username, username@domain, or username@domain.edu
- Passwords may not have the characters $! /
- Confirm that the credentials to access the folder have reading and writing permission.
- Nested folders may cause problems – try to set the Archive folder in the root of the server as shown in above example.
- A network load balancer may be an issue – contact Support@nanostring.com to discuss workarounds.

GeoMx Auxiliary Server

An auxiliary server provides additional computing power for the GeoMx DSP Data Analysis software. It is required for analysis of Cancer Transcriptome Atlas or Whole Transcriptome Atlas data. You can provide your own server or purchase a pre-configured server from NanoString. If providing your own, set up the server by installing its software according to GeoMx DSP Auxiliary Server Software Manual (MAN-10135), accessible in the software download folder, then pairing it with the GeoMx DSP following the steps below.

Ensure that there are no Data Analysis sessions in progress when configuring the auxiliary server; any sessions in progress will be terminated. Note that the GeoMx Data Analysis Auxiliary Server software must be updated each time the paired GeoMx DSP instrument software is updated.

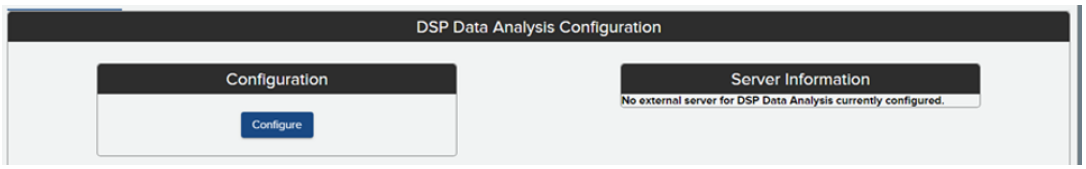


Figure 78: Auxiliary server configuration window before pairing

1. Select **GeoMx Auxiliary Server** from the **Admininstration** menu.
2. Click the **Configure** button (Figure 78).
3. Input the **IP Address** of the server. For a NanoString provided auxiliary server, this should be a static IP address; for a customer-provided auxiliary server, static or reserved DHCP is allowed. Click **Next**.
4. If the GeoMx system is able to identify the IP Address, you will receive a **successful** message. Click **Next**. (If the GeoMx system is unable to verify the IP address, you will be provided with the option to either cancel the configuration or try again with a new IP address.)
5. Enter the **six digit pin** provided to you during the installation of the **GeoMx Auxiliary Server** software. Contact Support@nanostring.com if you do not know this number. Click **Next**.
6. You will be asked to **confirm the configuration**. This is the last opportunity to abort the configuration. Click **Continue**. Data Analysis will be temporarily taken offline while setup continues.
7. When the **Setup Complete** window appears, click **OK**. The server availability and address will be displayed (Figure 79). If this information does not appear, refresh the screen (Ctrl + R).

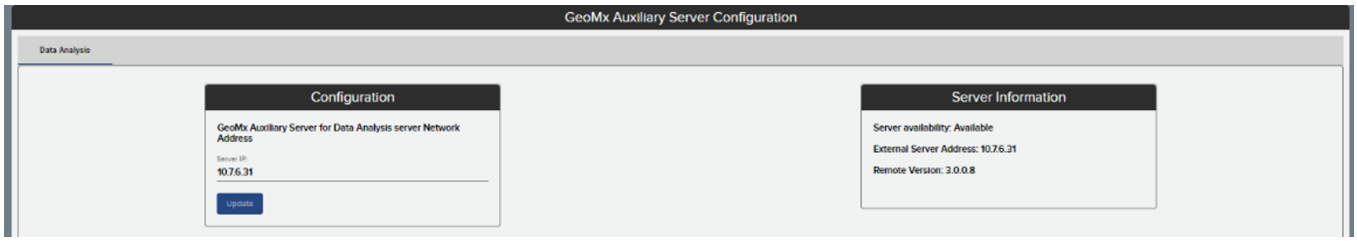


Figure 79: Auxiliary server configuration window after pairing

Group Management

> **IMPORTANT:** All Admin users are able to manage all groups.

In the GeoMx software you can create different user groups to control which users have access to data. In the Group Management tab, you can create a new group or edit an existing group (Figure 80).

Figure 80: Group Management tab under Administration

Creating a Group

1. Click **Create New Group**.
2. Enter a new Group Name and select **Save**.

Renaming a group

1. Click the **Rename** button next to the group of interest.
2. Enter the new name and select **Save**.

Deleting a group

1. Click the **Delete** button next to the group of interest.
2. **Confirm** to complete the deletion.

If deleting a group, that group's folders can be transferred to the **All Users** group. In **Records** right-click on the folder of interest and select **Edit**; the **All Users** group should be selected. Click **Save** to apply this change.

Managing Members

1. Click the **Manage** button next to the group of interest.
2. The group membership window will appear, listing the available users and the current members in the group (Figure 81).
3. To remove a user from the group, highlight the individual in the **Users in Group** field and select the **Remove Users** button.
4. To add an available user to the group, highlight them in the **Available Users** field and select the **Add Users** button.
5. Select **Save** to complete managing members.

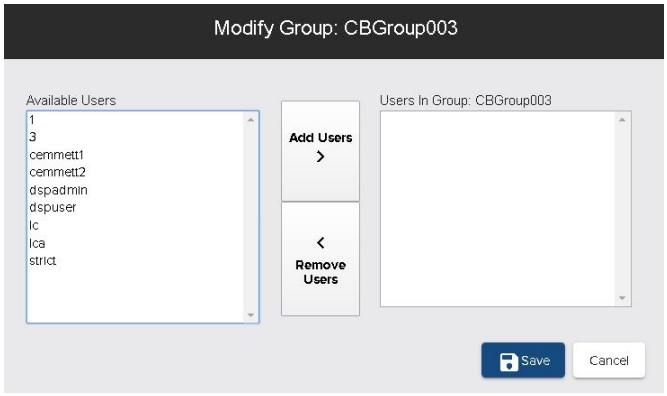


Figure 81: User group management window

Idle Timeout

Set the idle timeout settings for users. The default session timeout is 20 minutes (maximum 1439 minutes). The default reagent timeout (the duration that the Change Reagents prompt will remain on the screen when Change Reagents is offered mid-run) is 120 minutes (maximum 240 minutes).

Illumina BaseSpace Admin

This menu option is only accessible over a remote connection to the GeoMx DSP using the Chrome browser. Users may connect their GeoMx DSP to their Illumina BaseSpace workgroup(s) to enable seamless end-to-end workflows for experiments with NGS readout. These workflows allow a sequencing job to be sent from the GeoMx DSP directly to the BaseSpace Sequence Hub, eliminating the file processing steps of the conventional workflow.

Requirements:

- Use of Illumina NextSeq 1000/2000 sequencing platform
- Active DRAGEN subscription on the NextSeq 1000/2000

To add a connection to a BaseSpace Workgroup:

1. Access the GeoMx DSP over Chrome from a separate computer. Log in as an administrative user.
2. Click on **Administration,** then **Illumina BaseSpace Admin.**
3. Click **Add Connection to BaseSpace Workgroup.**
4. In the pop-up window (Figure 82), create an Account Name for this workgroup connection.
5. From the dropdown menu, select the region of your BaseSpace account (typically the region closest to your geographic location). **DO NOT click Connect yet.**
6. Open a new Chrome browser tab and navigate to the BaseSpace Sequence Hub. Log in to your account and select the Workgroup to which you wish to connect. **The same workgroup must be used** to log into the NextSeq 1000/2000 instrument and BaseSpace Sequence Hub. **Leave this browser tab open.**
7. Return to the GeoMx DSP browser tab and click **Connect to BaseSpace**. An authentication windows opens. Click Accept to grant permission for the applications to communicate.
8. Obtain the Illumina Platform API Key as follows, and enter it in the field of the pop-up window (Figure 82):

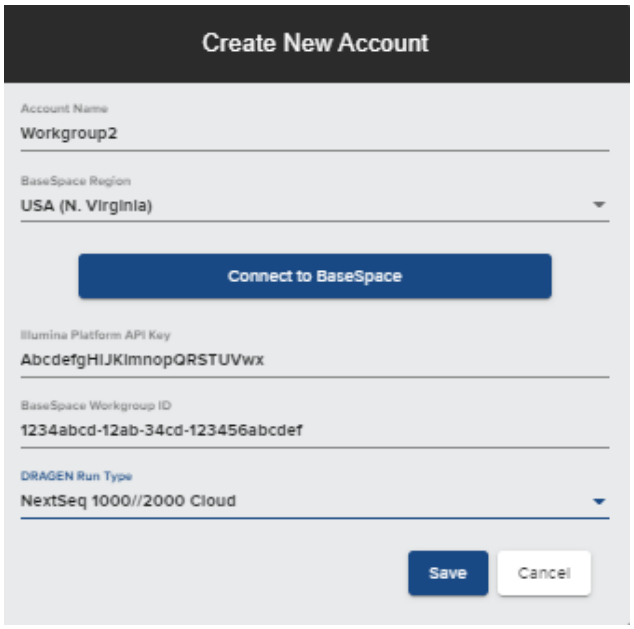


Figure 82: Illumina BaseSpace Admin pop-up

- In a third browser tab, navigate to https://accounts.login.illumina.com/platform-home/#/apiKey/list (Figure 83). (If your BaseSpace account is part of an Enterprise subscription, navigate to https://[YourEnterpriseDomain].login. illumina.com/platform-home/#/apiKey/list).

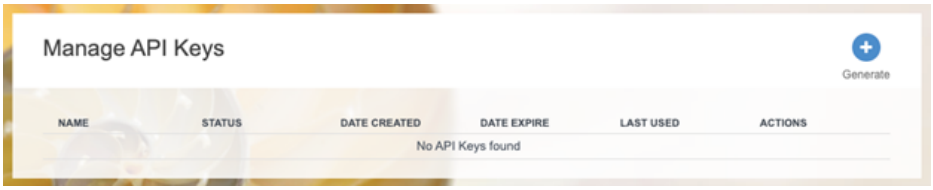


Figure 83: API Key List dialogue

- Click Generate to open the Generate an API Key dialogue (Figure 84) .

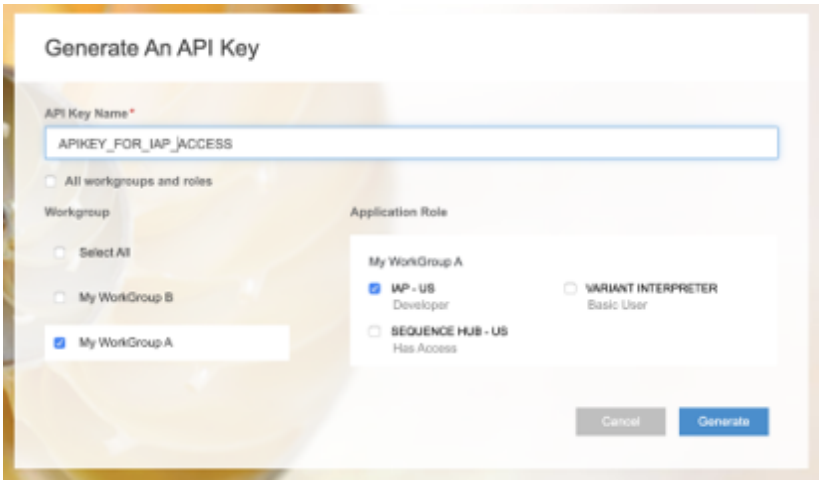


Figure 84: Generate an API Key dialogue

- Enter a name for the key. Un-check the box for "All workgroups and roles" - NanoString recommends generating a key for a specific workgroup. If your account is under multiple workgroups, choose the one with IAP permissions. Click Generate.
- A pop-up dialogue with the API key will display. Write down or download the API key.

  A user can only view or download the key at the time of generation. If the key is lost, a user must regenerate it. Keep the key in a secure location and do not share it with others, as such exposure may allow unknown users to access your data. If the key is compromised, a user should regenerate it and configure the GeoMx DSP connection with the new API key.

- Close the API Key Generated dialogue, and the newly generated key will appear in the key

list. Confirm that the key is set to “Never Expire”. Otherwise, the connection will be disrupted by token expiry during operation.

9. Obtain the BaseSpace Workgroup ID as follows, and enter it in the field of the pop-up window:

    - Open a fourth Chrome browser tab and navigate to https://api.basespace.illumina.com/v2/users/current/workgroups. (Enterprise users: navigate to https://api.[EnterpriseURL]/v2/users/current/workgroups - replace [EnterpriseURL] with the correct enterprise URL for your account). If you are outside of the United States, refer to the regional API URLs listed at https://developer.basespace.illumina.com/docs/content/documentation/cli/cli-overview#SpecifyAPIserverandAccessToken .

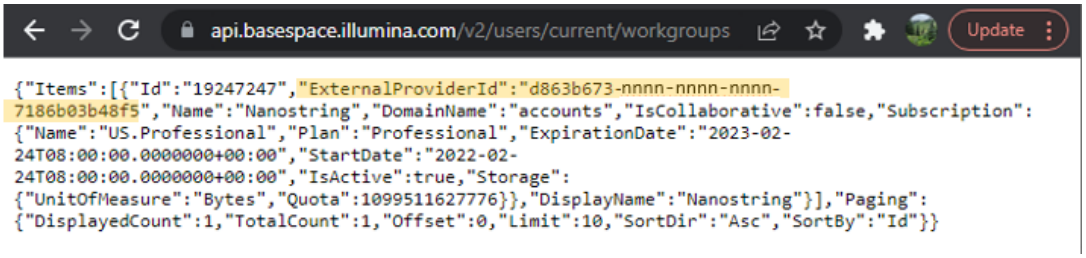


Figure 85: ExternalProviderID

    - A list of workgroups will load. Identify the workgroup to which you wish to connect **(must be the same workgroup as selected in Step 6, above)** and note your ExternalProviderID (Figure 85). Enter your ExternalProviderID in the BaseSpace Workgroup ID field of the pop-up window (Figure 82).

10. Enter the DRAGEN Run Type of your choice (Local or Cloud set-up).
11. Click **Save.**
12. See the connection listed in the Illumina BaseSpace Administration window of the GeoMx DSP. Click **Test** to confirm the connection or **Edit** to make changes if needed.

Kit Management

In the Kit Management tab (Figure 86) you can upload configuration files to your system.

Download configuration files from www.nanostring.com/dspconfigfiles, unzip them, and transfer **unzipped** files to the instrument via USB.

Use the check boxes to activate or inactivate kit configuration files. Only kits with checked boxes will be displayed in the Scan Configuration window.

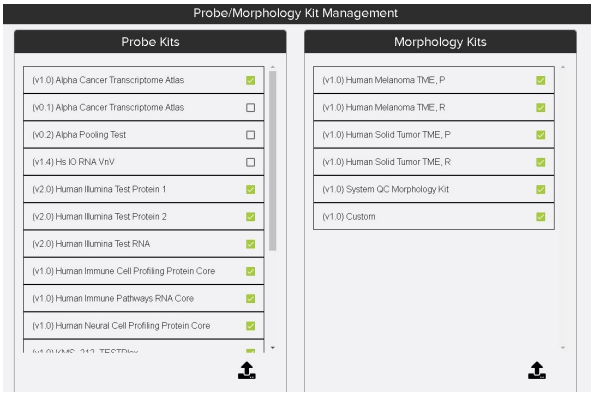


Figure 86: Kit file management window

Network Settings

The Network tab allows you to adjust network settings (Figure 87). Select **Dynamic** or **Static IP Address** from the dropdown menu.

> In the Network window, if **Machine Name** and **IP address** are not displayed, restart the system by shutting down from the System Settings tab, switching off the power switch on the back, waiting one minute, then switching power on.

Instructions to remotely connect to your DSP instrument

When connected to your system remotely, you can analyze data, select or modify ROIs, and export images.

1. Connect the GeoMx DSP instrument to your network with a network cable.
2. Log into the GeoMx DSP system with an administrative username and password.
3. Select the **Administration** button in the DSP Control Center.
4. From the Administration menu, select **Network**.
5. Note the **Machine Name** and the **IP address** listed in the **Network Settings** window.
6. From your remote computer, open a Chrome browser.
7. Browse to https://geomx-#### where #### is the GeoMx **Machine Name** you noted from the Network Settings window. In the example (Figure 87), the address is *https://geomx-B0008*.

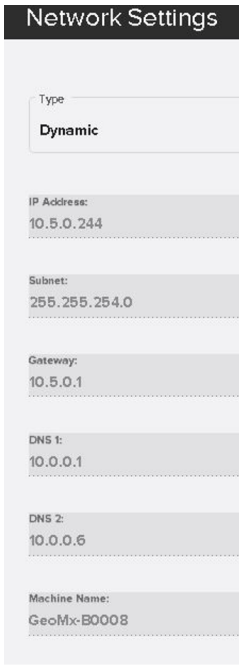


Figure 87: Network tab

8. If this does not work, your network may not pass on the GeoMx instrument name. In this case, enter the direct IP address. In the example (Figure 87), the address is *https://10.5.0.244*.
9. This should return the GeoMx DSP login screen, where you can log in with your user account. You will be able to perform data analysis, select ROIs, and export images.

Closing the Chrome browser will automatically log you out of the GeoMx system.

Performance Monitoring

The GeoMx Instrument Performance Monitoring Program securely and remotely collects **instrument performance** information, allowing the service and support teams to automatically access logs and troubleshoot when needed. This access may also be used to analyze run metrics, understand trends, and proactively make improvements. **Importantly, performance monitoring only collects instrument performance data and not biological or experimental data.** The following information is **not** collected: images, sample or slide names and annotations, morphology marker information, probe kit information, or ROI or segment names and annotations. A continuous internet connection is required to enable performance monitoring.

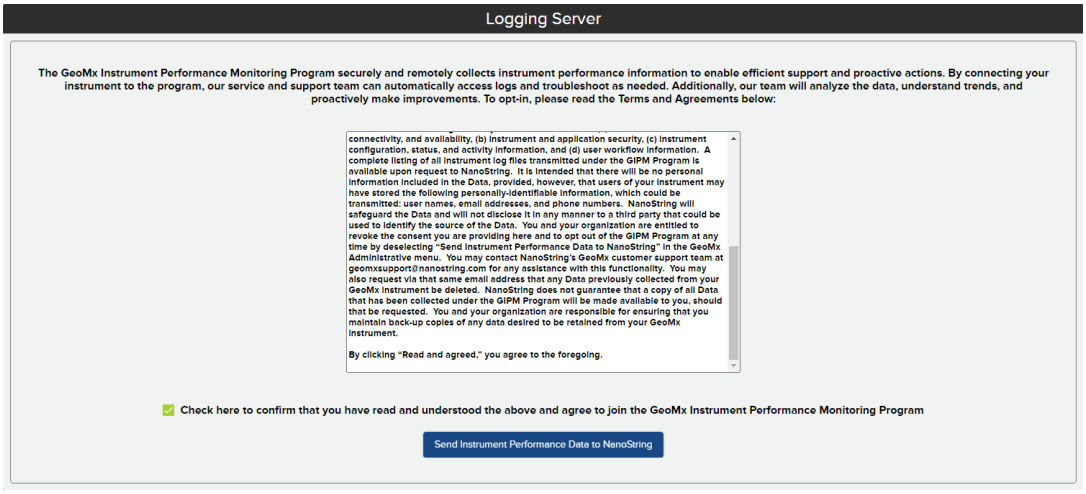


Figure 88: Performance Monitoring option

To turn on performance monitoring:

1. Select **Performance Monitoring** from the **Administration** menu (Figure 88).
2. Read the **Terms and Agreements** on this page, scrolling to the end.
3. Check the box confirming you have read and agree to the terms.
4. Click the **Send Instrument Performance Data to NanoString** button.

5. A message will appear, indicating a successful enrollment.
6. Restart the GeoMx DSP instrument to begin performance monitoring.

To turn off performance monitoring:

1. Select **Performance Monitoring** from the **Administration** menu.
2. Click the **Opt-out of Performance Monitoring Programming** button.
3. A message will appear, indicating a successful disabling of performance monitoring.

Platforms

Use the Platform Selection window (Figure 89) to indicate the platforms your laboratory uses for GeoMx DSP data readout: nCounter, Illumina NGS, or both.

Figure 89: Platform Selection window

SSL Settings

This is only relevant for computers used to remotely access the GeoMx DSP interface. This process is not necessary for the on-board DSP computer.

If your browser does not recognize the GeoMx DSP software security certificate and warns that the site is "not secure", follow your browser's prompts to proceed anyway. Follow the instructions below to download and install the security certificate. Certificates expire periodically; you may need to repeat this procedure to download and install an updated certificate again in the future.

Figure 90 : SSLSettings

Installing your GeoMx instrument's unique self-signed root CA certificate

1. **Login** to the GeoMx system as an administrative user. Click on the **Administration** button and select the **SSL Settings** button from the menu.
2. The SSL Settings window opens (Figure 90). Click **Download** to download the security certificate. If prompted, browse to the preferred download location. You may see a warning at the bottom of your browser window: *This type of file can harm your computer. Do you want to keep systemca.crt anyway?* Click **Keep**.
3. Use a USB drive or your network to **transfer the certificate file to your remote computer** and save it in an accessible location.
4. **Navigate to the certificate**, double click on it, and select **Open.**
5. In the Certificate window, click the **Install Certificate** button.
6. **Choose Local Machine** and click **Next**. Administrative privileges are required for this step; you may need to contact your IT department.

7. Select the option for **Place all certificates in the following store**, then click **Browse** (Figure 91).
8. **Browse** to **Trusted Root Certification Authorities** and click **OK** (Figure 92).
9. Complete the remainder of the **Certificate Import Wizard** steps until you receive a successful message.
10. Close any open tabs on Google Chrome, then relaunch the browser. You should now be able to connect to the GeoMx system remotely. To do this, browse to https://geomx-#### where #### is the GeoMx Machine Name (find this information under the **Network** option under **Administration**).

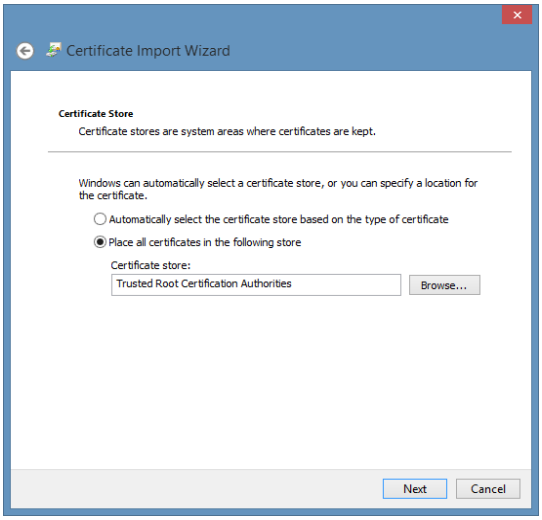


Figure 91: Place all certificates...

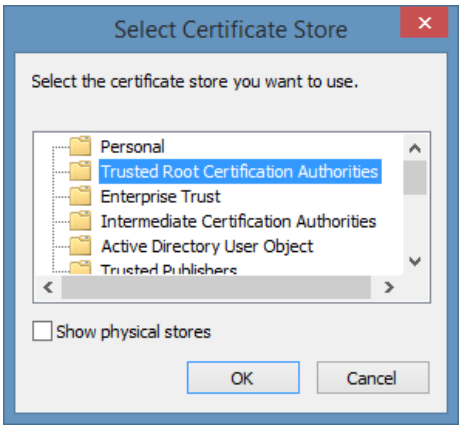


Figure 92: Trusted Root Certificate Authorities

See also Configure and Install SSL Certificates for GeoMx DSP (MAN-10168).

Scan Parameters

Create and manage records for fluorophores and biological targets to be available for custom analysis in the Scan Configuration window during scan set-up (Figure 93).

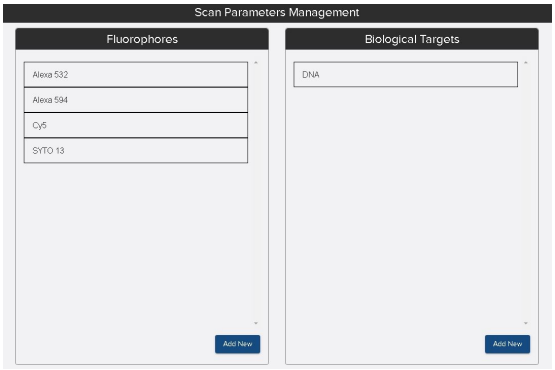


Figure 93: Scan Parameters management window

Software Update

Refer to the software version-specific installation instructions. These are released with each new GeoMx DSP Instrument software version.

> **IMPORTANT:** If your GeoMx is configured to use an auxiliary server, it is required to update the NanoString software on the auxiliary server as soon as the GeoMx DSP software update is complete. See the GeoMx Auxiliary Server Software Installation Manual (MAN-10135), accessible in the software download folder. Until the auxiliary server software is updated, studies will not be able to be opened or created.

Bring GeoMx DSP Software to an Idle Software State

There are two points in the workflow where the software is in an idle state: **Replace Plate** and **Collection Complete**. If not already in an idle software state, do the following:

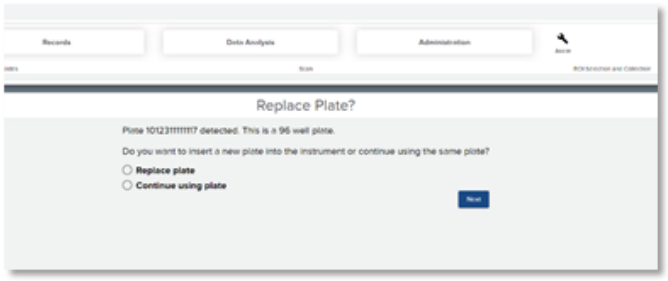


Figure 94: Replace Plate idle state

1. Click the **New Data Collection** button or the **Data Collection** tab, then **New/Continue Run**. If these buttons are not available, click **Assist** (wrench icon), then **Restart Data Collection Workflow**.
2. If there is a plate in the instrument, you'll see **Replace Plate?** (Figure 94). Pause here (do not click Next) – this is an idle software state.
3. If there is not a plate in the instrument, you'll be prompted to load one. The slide holder should be empty of slides and loaded in the instrument.
4. Wait for pop-up message "**No Slides Detected**". Click **OK.** Now, the instrument is at the Collection Complete idle software state (Figure 95).
5. Click **Administration**, then **Software Update** to perform the update.
6. Refer to the software version–specific installation instructions. These are released with each new GeoMx DSP software version.

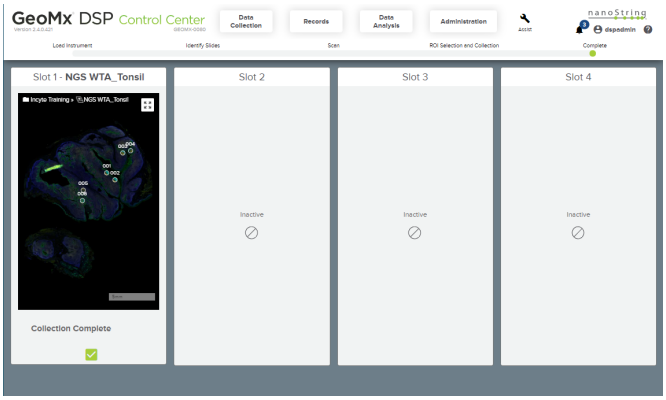


Figure 95: Collection Complete idle state

System Backups

Once an administrative user has provided a network path for archiving (see Administration Menu on page 77), the system will automatically start archiving data and performing backups. The dates and location of system backups will be listed here. The list may not include all successful system backups; check the external archive location to view all archived files.

System Settings

Through the System Settings menu, you can shut down the instrument (see Shut Down on page 102) or change the system time.

Your GeoMx instrument (and, if applicable, the paired auxiliary server) rely on having accurate date and time settings to establish secure connections. NanoString recommends setting the internet time server as follows:

1. Log into the GeoMx DSP system as an administrative user.
2. From the Administration menu, click on **System Settings**, then **System Time**. The **System Time** window will open (Figure 96).
3. Check the box for **Synchronize with Internet Time (NTP) Server**.
4. Enter **ntp.ubuntu.com** into the Time Zone Server field.
5. Select the appropriate time zone for your location.
6. Click **Save Changes**.

If the dialogue box indicates the time server is **Local CMOS Clock**, then the specified time server cannot be reached. Check with your network administrator to allow outbound network traffic from your GeoMx DSP and auxiliary server to the address ntp.ubuntu.com. If it's not possible to use ntp.ubuntu.com, select an alternative time server that you can successfully connect to.

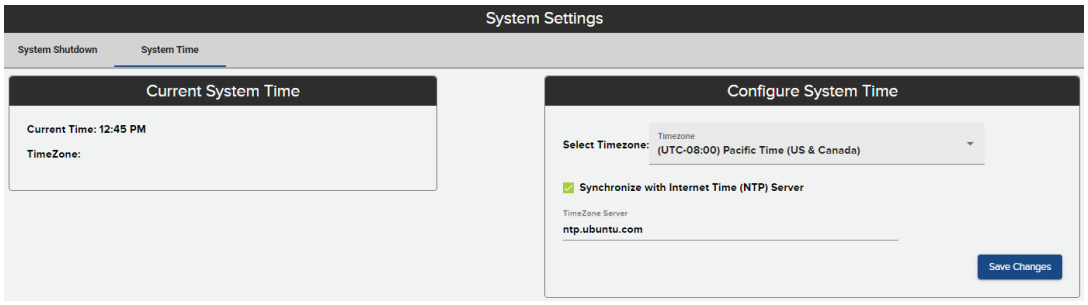


Figure 96: System Settings - System Time

User Management

The **User Management** window allows you to edit or create new user profiles (Figure 97). You can also manage the type of password users are allowed to use. Scroll to the bottom of the **User Accounts** list and check the **Enforce Strict Passwords** box to require users to have more complex passwords. With this box checked, each user password created from this point forward must be at least 14 characters, containing at least three of the following: uppercase letter, lowercase letter, number, and special character.

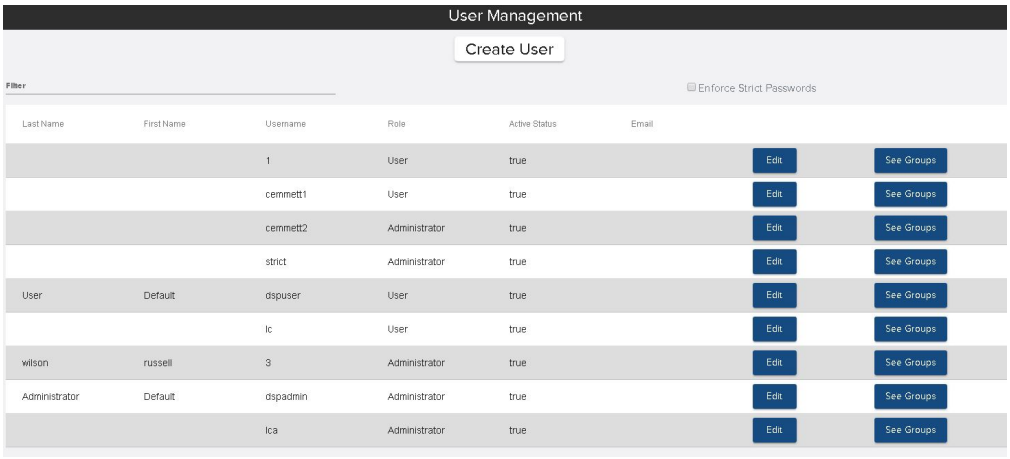


Figure 97: Manage Users tab under Administration

Edit User

1. On the **User Management** tab under **Administration**, locate the profile you would like to edit in the user list and select the **Edit** button in that row.
2. Edit the fields as needed.
3. Select **Save.**

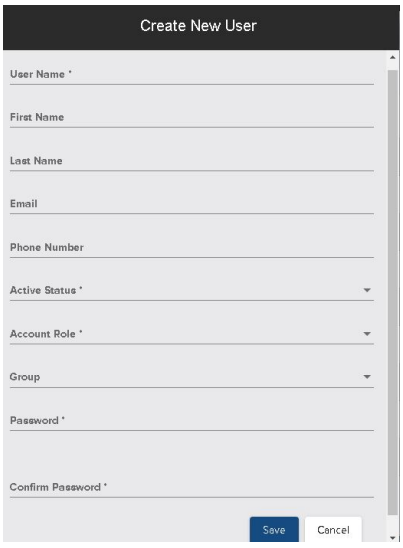


Figure 98 : Add User

Add User

1. On the **User Management** tab under **Administration,** select **Create User**.
2. The **Create New User** window will appear (Figure 98); fill out the required fields (indicated with *).
   - Create a username and password for the new user. Do not use spaces in usernames.
   - Active status can be **Active** or **Inactive** (inactive users will not be able to log into the system).

- Account Role can be set to **Administrator** or **General** (general is default; general users do not have access to most of the Administrative menu options). In order to change a user's role (Admin to User, for example), that user must be assigned to no more than one group.

  Consider establishing more than one Administrator at your workplace so that you have at least one backup if the primary Administrator is unavailable to perform administrative tasks.

- Enter the user's e-mail address, phone number (if desired), and name.

3. Select **Save**.

If a user creates a folder with data, then the user is made inactive or is removed from all groups, the folder will still be accessible by Admin level users of the system.

Warning Reset

If the GeoMx System detects a potential problem, it may issue a **Warning Symbol** on the footer of the GeoMx DSP Control Center (Figure 99) . Clicking on this symbol reveals any codes associated with the current state of the system. If encountering a warning on your system, contact Support@nanostring.com. At the instruction of NanoString Technical Support, you may reset the warning light by navigating to **Warning Reset** under **Administration** and clicking the **Reset** button.

Figure 99: Warning symbol

GeoMx DSP Instrument Reagents

Figure 100: GeoMx DSP instrument reagent bay diagram

The GeoMx DSP instrument requires the following reagent bottles in the reagent bay (Figure 100):

- **Buffer S**: 1 L bottles in reagent bay slots 1 and 4.
- **Buffer H**: 250 mL bottles in reagent bay slots 2 and 3.

In addition, the **waste bottle**, with adequate space to collect waste, must be present in the position to the right of the reagent bottles.

IMPORTANT: Always use the **Change Reagents wizard** and exchange full bottles. DO NOT top off bottles, or exchange bottles without the Change Reagents wizard.

IMPORTANT: Do not open the reagent bay door during a run, unless prompted by the system to change reagents. Opening the door may interrupt and abort the current run.

Changing Reagent Bottles

The GeoMx DSP system tracks reagent usage and waste levels and displays the volume of each in the **Reagent Status Indicator** in the lower right of the Control Center (Figure 101).

The system checks reagent levels before ROI collection on each slide; if a bottle runs too low for collection, you will be prompted to replace the depleted bottle.

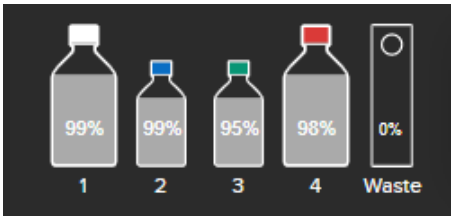


Figure 101: Reagent status indicator

To change the reagents at another time, launch the Change Reagents wizard by clicking the **Reagent Status Indicator.** This can be launched at any time during the workflow and the system will pause at a suitable time for reagent changes. Follow the prompts by the GeoMx DSP system:

1. Only when prompted by the system, open the reagent bay door. Slide the reagent tray out for better accessibility.
2. Remove the fitting from the bottle cap by pinching the dark gray button on the QuickConnect with thumb and forefinger (Figure 102).
3. Unscrew the cap and remove it.
4. Remove the used bottle and replace it with an uncapped full bottle with the appropriate buffer.
5. Screw the cap back on and reattach the fitting, continuing to press even after the first 'click' to ensure that the fitting is secure. The gray button will pop out when the bottle is secure.

Figure 102: Disconnecting GeoMx reagent bottle

> **IMPORTANT:** Use clean gloved hands during this process. Ensure that the bottles are securely connected to their fittings after exchanging reagents or emptying waste. A connection that is not secure may leak and cause damage to the instrument.

6. Gently push the reagent tray back in and close the door.

After the wizard steps are complete, the system updates reagent levels in the Reagent Status Indicator.

Emptying the waste

The system will check the waste level periodically; if the waste is too full, you will be prompted to empty it. You can also click on the **Reagent Status Indicator** in the GeoMx DSP Control Center to initiate the waste emptying process if the instrument is in an idle software state (see Bring GeoMx DSP Software to an Idle Software State on page 94).

1. When prompted by the system, open the reagent bay door. Slide the reagent tray out for better accessibility.
2. Remove the fitting from the waste bottle cap by pinching the dark gray button on the QuickConnect with thumb and forefinger (Figure 103).

Figure 103: Disconnecting GeoMx DSP waste container

3. Remove the waste bottle from the reagent bay holder (Figure 104).
4. Unscrew and remove the cap assembly from the waste bottle.
5. Empty the waste bottle.
6. Rinse the waste bottle several times, eliminating all foam. Residual bubbles can falsely trigger the Waste Full sensor.
7. Screw the cap back on, insert the waste bottle into the reagent bay of the instrument, and reattach the fitting, ensuring that the QuickConnect clicks in tightly.

Figure 104: Removing GeoMx DSP waste

> **IMPORTANT:** Use clean gloved hands during this process. Ensure that the bottles are securely connected to their fittings after exchanging reagents or emptying waste. A connection that is not secure may leak and cause damage to the instrument.

8. Gently push the reagent tray back in and close the door.

GeoMx DSP Instrument Maintenance

System Startup & Shutdown

The GeoMx DSP instrument should be shut down and restarted **at least weekly** as well as before every new run. The power button is located on the back of the instrument (Figure 105).

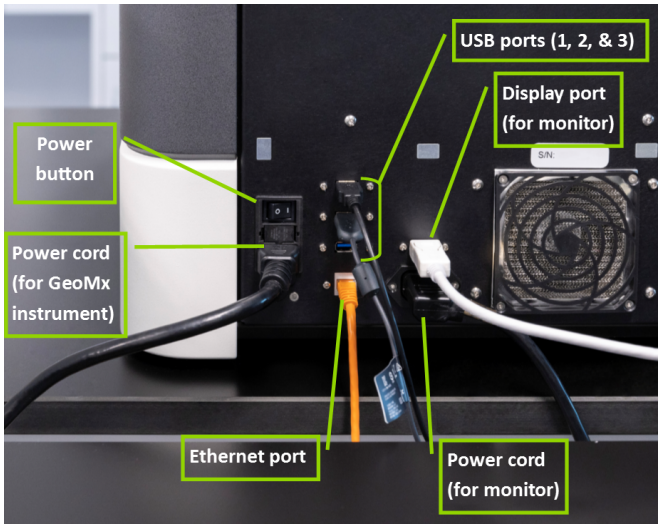


Figure 105: GeoMx DSP backside diagram

Start Up

1. Switch the GeoMx DSP instrument power button to the **On** position. The hardware will initialize, homing and resetting positions as necessary.
2. Once the system has initialized, the DSP screen and a login window will appear on the monitor.
3. Once you have logged in, navigate to **Data Collection, Records, Data Analysis,** or **Administration.** To begin a new run, click the **New Data Collection** button or **Data Collection, New/Continue Run**. If these buttons are not available, click **Assist** (wrench icon), then **Restart Data Collection Workflow**.

Shut Down

The GeoMx DSP instrument should be shut down and restarted at least weekly.

IMPORTANT: Leave the slide holder in the instrument. Ensure it is in the instrument before shutting down or restarting.

1. A user must be logged in locally in order to shut down the instrument.
2. Select the **Administration** button from the GeoMx DSP Control Center.
3. Select **System Settings**, then the **System Shutdown** tab, then **Shutdown System**.
4. The system will determine if any slide runs are in progress.
   - If one or more users are logged in, a warning message will appear. Select **OK.**
   - If no slide run is in progress, the system will warn that all users will be logged out and may lose unsaved information. Select **OK** to continue shutting down.
5. A message will instruct you to allow 30 seconds before turning the physical power button to the **off** position. Select **OK** to continue shutting down.
6. A status message will indicate that system shutdown is in progress. The system will perform any hardware functions required to prepare for long-term storage, homing if necessary.
7. The system will power off.
8. Once the monitor turns black and indicates “no signal," turn the instrument power button **off**. Wait at least 1 minute before turning on again, if rebooting.

   The fans of the system may continue to blow after the system has been shut down. If the monitor has turned black, the computer inside the system has shut down and the hardware of the system is in the home position. Despite the fans still operating, it is safe to turn the power button off at this stage.

Cleaning the Slide Holder

Clean the slide holder after each run to ensure proper instrument function.

1. If neccessary, remove buffer from loaded slides with a pipette and then remove slides from the slide holder.
2. Rinse the slide holder thoroughly with distilled water from a squirt bottle. Gently pat dry with Kimwipes.
3. Inspect the entire slide holder. Ensure that the springs of each slide clamp and the gasket (blue rubber) of each slide slot are clear of debris from salt, dust, or lint.
4. If necessary, gaskets may be removed for thorough cleaning with distilled water. Allow to air dry or pat dry with Kimwipes. Inspects for tears or damage. Carefully replace gaskets to slide holder, ensuring a good fit.

   To minimize wear and tear on gaskets, avoid frequent removal from the slide holder. A brief cleaning following each instrument run should keep the gaskets clean without needing to remove them from the slide holder.

5. Visually inspect the digital micromirror device (DMD) calibration target (Figure 106) to ensure it is clear of all debris. Do not contact the target unless necessary to gently wipe away debris. DO NOT apply pressure to the target.
6. Return the slide holder to the GeoMx instrument between runs.

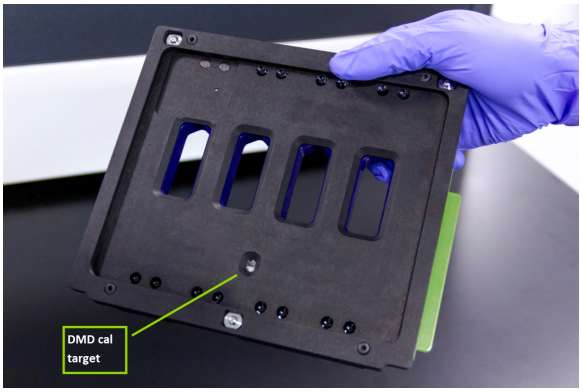


Figure 106: Underside of slide holder with DMD calibration (DMD cal) target indicated

IMPORTANT: If the slide holder has been dropped, its performance could be compromised. Run a test slide before using the slide holder with experimental samples. Contact Support@nanostring.com for more specific guidance.

Hibernating the GeoMx DSP for Extended Shutdown

If the GeoMx DSP instrument will be inactive for 2 weeks or more, NanoString recommends following this hibernation protocol to flush the fluidic lines of instrument buffers and fill the lines with DEPC-treated (or other nuclease-free) water. When desired, the system can be returned to operational as described below.

Materials:

- Two 1L bottles of DEPC-treated or other nuclease-free water. Note: Thermo Fisher AM9922 Ambion DEPC-Treated Water (1L) has the correct threading to match the Buffer S bottle tops. An alternative to commercial DEPC-treated water is DEPC-treated water prepared in-house, following standard lab protocols.
- 1 spare tissue slide (for example, the tissue slide used in GeoMx training). A blank slide with a small indelible pen mark in the center and in the label area may suffice.
- 1 spare collection plate.

Hibernate the system:

1. Switch Buffer S bottles to DEPC-treated water bottles. Perform these steps **without** launching the Change Reagents wizard:
   a. Open the bottle bay door and unscrew the bottle cap assemblies from the Buffer S bottles in bottle slots 1 and 4.
   b. Replace these two bottles with DEPC-treated water. Close the bottle bay door.
   c. Cap and save your bottles of Buffer S to use when returning the system to operational.
2. Prime lines:
   a. From the **Administration** menu, select **Admin Scripts** (you must be logged in as an administrative user). From the dropdown menu, select **Admin_SystemPrimeFromBottle**. In the field for Parameter Value, enter **1**. Click **Proceed**. The instrument primes the Bottle 1 line (allow 8 minutes to complete).
   b. Leave the Admin Scripts page, then select **Admin Scripts** again. Enter Parameter Value of **4**. Click **Proceed**. The instrument primes the Bottle 4 line (allow 8 minutes to complete).
3. Begin a mock run to push water through fluidic lines:
   a. Start a new run by clicking **New Data Collection**, or **Data Collection** then **New/Continue Run**. If these buttons are not available, click **Assist** (wrench icon), **Restart Data Collection Workflow.** When prompted:
      i. Insert slide holder with a spare slide in slot 1, covered with DEPC-treated water.
      ii. Insert the spare collection plate.
      iii. Select any Morphology Marker kit from the dropdown menu. **Do not select any Probe Kit**. The system will warn that you will not be able to collect from this slide.

iv. Proceed through Preparing Instrument and Initializing Fluidics to generate a low magnification scan.

b. Define a scan area in the low magnification scan to proceed to the high magnification scan.

c. When the high magnification scan is complete, the system will initiate cleaning and move to the **Collection Complete** idle software state.

d. Once at the **Collection Complete** idle software state, click on **Remove Slides and Microplate**.

e. When prompted, open the door to retrieve the slide holder. Store the slide, thoroughly clean the slide holder, and return the holder to inside the instrument.

f. Remove the collection plate and keep as a spare.

g. When finished, close the door to the instrument.

4. Turn off the instrument (optional):

a. Select the **Administration** tab from the GeoMx DSP Control Center.

b. Select **System Settings**, then **System Shutdown**, then **Shutdown System**.

c. When the screen turns black (after ~30 seconds), power off by flipping the switch on the back-right of the instrument.

Return the system to operational:

1. Switch DEPC-treated water bottles to Buffer S bottles. Perform these steps **without** launching the Change Reagents wizard.

a. Open the bottle bay door and unscrew the bottle cap assemblies from the DEPC-treated water bottles in slots 1 and 4.

b. Replace these two bottles with the Buffer S bottles that were removed previously. Close the bottle bay door.

2. Power On the instrument, if it was off:

a. Flip the switch on the back-right of the instrument to On. The system will boot up, which may take a few minutes.

b. Log in to the Control Center using an administrative user account.

3. Prime lines:

a. From the **Administration** menu, select **Admin Scripts**. From the dropdown menu, select **Admin_SystemPrimeFromBottle**. In the field for Parameter Value, enter **1**. Click **Proceed**. The instrument primes the Bottle 1 line (allow 8 minutes to complete).

b. Leave the Admin Scripts page, then select **Admin Scripts** again. Enter Parameter Value of **4**. Click **Proceed**. The instrument primes the Bottle 4 line (allow 8 minutes to complete).

4. Begin a mock run to push buffer through fluidic lines:
   a. Start a new run by clicking **New Data Collection**, or **Data Collection** then **New/Continue Run**. If these buttons are not available, click **Assist** (wrench icon), **Restart Data Collection Workflow.** When prompted:
      i. Insert slide holder with a spare slide in slot 1, covered with Buffer S.
      ii. Insert the spare collection plate.
      iii. Select any Morphology Marker kit from the dropdown menu. **Do not select any Probe Kit**. The system will warn that you will not be able to collect from this slide.
      iv. Proceed through Preparing Instrument and Initializing Fluidics to generate a low magnification scan.
   b. Define a scan area in the low magnification scan to proceed to the high magnification scan.
   c. When the high magnification scan is complete, the system will initiate cleaning and move to the **Collection Complete** idle software state.
   d. Once at the **Collection Complete** idle software state, click on **Remove Slides and Microplate.**
   e. When prompted, open the door to retrieve the slide holder. Store the slide, thoroughly clean the slide holder and return the slide holder to inside the instrument.
   f. You may remove the spare collection plate (and save for similar uses).
   g. When finished, close the door to the instrument.

If the instrument was kept on during the hibernation, it is recommended to restart it before the next run.

General Cleaning

Follow all safety and operating instructions provided in this manual. Use safe laboratory operating precautions, including use of personal protective equipment such as safety glasses and gloves.

- Always ensure the waste bottle has sufficient capacity before starting a new run.
- If a spill occurs, clean the area by wiping with a disinfectant followed by wiping with water or **70% ethanol**. A disinfectant such as DNAZap™ from Invitrogen is recommended. Use a damp towel rather than spraying directly on the instrument.
- Clean the exterior of the instrument using a diluted neutral soap, followed by water. Use a damp towel rather than spraying directly on the instrument.

Replace Air Filter

ELECTRICAL HAZARD: Do not attempt to disassemble the instrument at any time. An electric shock can occur if the instrument is operated without its outer case. Properly shutdown, then disconnect the instrument from the power source before attempting to replace the air filter.

The air filter located on the back of the instrument will need occasional replacement, typically every 12– 18 months. If the filter appears to be visibly obstructed by debris, contact Support@nanostring.com to request a replacement.

To replace the air filter:

1. Lift up on the filter chassis to remove it from the instrument.
2. Unscrew the cover by hand (do not use tools).
3. Remove the metal cover.
4. Remove and dispose of the mesh filter. Do not attempt to clean and re-use the filter.
5. Insert the new filter supplied by NanoString.
6. Replace the metal cover and affix the screw by hand (do not use tools).
7. Return the filter chassis by lowering it into the instrument.

Instrument Best Practices

- Reboot instrument prior to beginning an instrument run, and at least weekly.
- To properly shut down the instrument, select **Administration, System Settings** then **Shutdown**. When the screen goes dark, flip the switch at back of instrument to Off.
- While the instrument is powered down, open the Reagent Bottle Bay and disconnect and re-secure QuickConnect cap fittings on all reagents and waste bottle. Do not exchange reagents outside of the Change Reagents wizard.
- Don't overtighten the waste bottle cap. It's recommended to screw the cap on until it is just barely tight, then loosen it about 1/8 of a turn.
- To begin a new run, click the **New Data Collection** button, or **Data Collection** then **New/Continue Run**. Only if these buttons are not available, click **Assist** (wrench icon) then **Restart Data Collection Workflow** to bring the software to the workflow starting point.
- Leave the clean, dry slide holder inside instrument at the end of a run.
- Follow hibernation protocol if instrument will not be used for ≥2 weeks.

Troubleshooting

If you run into a problem with the instrument, try these steps:

1. Click F5 or click Assist (wrench icon) then Refresh to update the screen view.

2. If the problem is not resolved, log out by pressing Ctrl Alt Del then Sign Out. When the screen shows the time, click anywhere to return to the users screen. Then sign in to Kiosk User.

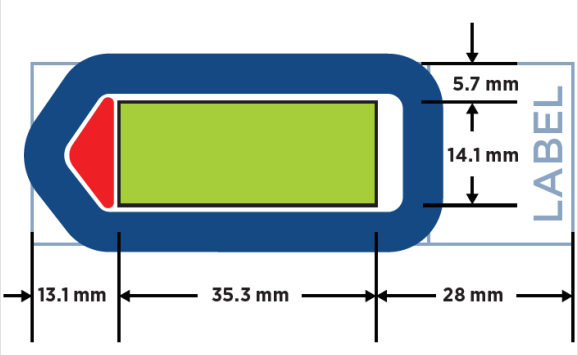
3. If the problem is not resolved by F5 or logging out:

- If you are NOT in the middle of a run, reboot the instrument by selecting **Administration, System Settings** then **Shutdown**. When the screen goes dark, flip the switch at the back of instrument to off. After 1 minute, flip it back to on. Upon logging in, click **New Data Collection**, or Data Collection then **New/Continue Run**, to begin a run. If these buttons are not available, click **Assist** (wrench icon) then **Restart Data Collection Workflow.**
- If you ARE in the middle of a run, contact Support@nanostring.com to open a support case.

Suggested actions to resolve certain issues are listed below (Table 5).

Table 5: Troubleshooting

Issue	Possible cause	Suggested action
Screen did not update or software is non-responsive	Lag in the user interface	Click **F5** on keyboard or **Assist** (wrench icon), then **Refresh page**. If not resolved, log out **using Ctrl Alt Del** then Sign Out. Then sign in to Kiosk User and log back in. If neither option succeeds, follow guidance under Step 3, above.
Can't log in to instrument	Internal clocks are out of sync	Click **Ctrl Alt Del** then Sign Out. Then sign in to Kiosk User to attempt to log in again. If not resolved, reboot the instrument (if you can't log in to reboot through the software, perform a hard-power-off by flipping the switch on the back, wait 1-2 minutes, then turn back on). Do not turn off instrument while in the middle of a run. If presented with on-screen notification about internal clocks, contact Support@nanostring.com to help reset clocks.
Can't log in from a remote computer	Clocks are out of sync	Check that the remote computer clock and the GeoMx DSP instrument clock are set to the same time.
The software prompts to	The latch may be sticky	Press down and slightly to the left to release the latch in the lock mechanism. If the problem is reoccurring, contact Support@nanostring.com

Issue	Possible cause	Suggested action
open the door, but it is locked		to arrange for field service.
	Lag in the software	Refresh the screen. Log out and log in again. If not resolved, click **Assist** (wrench icon), **Restart Data Collection Workflow**; then **Data Collection, New/Continue Run** to go to the start of the workflow. On-screen prompts to load the instrument should coincide with unlocking the door, allowing you to unload or load the instrument.
Slides are not recognized by the instrument	Tissue may be outside scan area, impeding camera detection	Confirm that tissue is within the Scan Area boundary (shown in green in Figure 107 - measure from label end of slide as reference edge). Scrape away excess hydrophobic pen residue, if needed, with a razor blade. Load slides again.  Figure 107: Scan Area in green (not to scale)
	Blank scan label area may be impeding camera detection	Make a mark on the slide label area with a black indelible marker and load slides again.
Fluidics error following Change Reagents	QuickConnect bottle cap fittings are not secure	Confirm that all QuickConnect cap fittings are secure. Continue pressing after the first “click” when securing the fittings, until QuickConnect button pops out. For best practice, secure all QuickConnect fittings each time the instrument is off. If failing at bottle check, follow on-screen prompts to retest bottle connection.

Issue	Possible cause	Suggested action
Accidentally ran experiment with wrong set of probe kit configuration (.pkc) files selected	User error	See instructions on amending .pkc after the experiment on page 71 .
Scan transfer error: No imagery found for transfer	Selected scan is not eligible for transfer	Select a scan for transfer that has status: Scanned, Aborted, Error, Collecting, Collected, Awaiting Readout, or Readout Complete.
I can't get ROI segments to look the way I want them	Overcomplicated segmentation strategy	A complex segmentation strategy may lead to ROI segments that don't meet your expectations by eye. In other words, you can't get the segments to capture the cell regions you wish to capture. In this case, it's recommended to try a very basic segmentation strategy - for instance, each segment with one definition (e.g. +PanCK). A simple strategy may produce segments that better capture the cells of interest.
After approving ROI, instrument does not proceed to collect	Root cause can only be determined from log files, but it is often due to tissue extending into tip calibration area of slide, or low volume of Buffer H or S	Contact Support@nanostring.com to evaluate the situation. This issue can often be prevented by examining slides to ensure that tissue does not exceed Scan Area boundary (Figure 107); wiping the bottom of each slide clean with 70% ethanol and a Kimwipe before the instrument run, and ensuring Buffer H and S have 20% volume or more prior to starting a run.
After filling one plate, instrument does not prompt to change plates	Lag in the user interface	Log out of GeoMx DSP by clicking the username at top right, then Log Out. Log back in.
Plate status indicator shows	Temporary hardware or	Typically, the hardware or software recover and proceed with collection. For assistance, contact Support@nanostring.com.

Issue	Possible cause	Suggested action
a red well during collection	software error caused a skipped segment or ROI	
For nCounter Readout: Collected into the wrong row, or I don't have HybCode to match the collection row.	User error	The collection row must match the Hyb Code letter (A, B, C, etc). You may re-scan the slide(s), selecting different ROI, and collect into plate rows that match the Hyb Code you have on hand, or order additional Hyb Code to match the row of the collected aspirates. Contact Support@nanostring.com for further guidance.
Screen is stuck on the pop-up window "Unload instrument"	Software is lagging	Click **Ctrl Alt Del** then **Sign Out**. When the screen shows the time, click anywhere. Sign in to Kiosk User.
	Plate cover is still covering the plate; instrument has not detected unloading.	The plate cover must be moved all the way to the right. If the door is locked, you may need to power-off the instrument, open the door when the instrument is off and slide the plate cover to the right, close the door, then restart the instrument.
I need to get my slides and plate out	Error in run or time constraints	If available, select **Remove Slides and Microplate,** or **Data Collection** then **New/Continue Run**. (If not, click **Assist** (wrench icon), **Restart Data Collection Workflow**). At **Replace Plate?** screen, select **Replace plate** and proceed through instrument prompts to open the door, or pause at **Replace Plate?** screen and navigate to **Administration**, **System Settings**, **Shutdown System**. Wait ~30 sec until screen is dark, then turn Off via switch on back of instrument. Once powered off, door is unlocked to access your samples.

Symbols & Definitions

	Manufacturer
	Authorized Representative in the European Community (safety)
	Safety CE Mark
	UK Conformity Assessed Mark
	Catalogue or Reference Number
	Batch code / Lot number
	Serial number
	Temperature range storage conditions
	Lower limit of temperature storage conditions
	Upper limit of temperature storage conditions
	For Use by / Expiry Date

NanoString Technologies, Inc.
530 Fairview Ave North
Seattle, Washington 98109 USA
www.nanostring.com

CONTACT US
info@nanostring.com
Tel: +1 888 358 6266
Fax: +1 206 378 6288

SALES CONTACTS
United States: us.sales@nanostring.com
EMEA: europe.sales@nanostring.com
Asia Pacific & Japan: apac.sales@nanostring.com
Other Regions: info@nanostring.com