NANOSTRING USER MANUAL

GeoMx - nCounter
DSP Instrument
User Manual

CE

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Conventions

The following conventions are used throughout this manual 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.

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

IMPORTANT: This symbol indicates important information that is critical to ensure 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.

GeoMx DSP Instrument Introduction

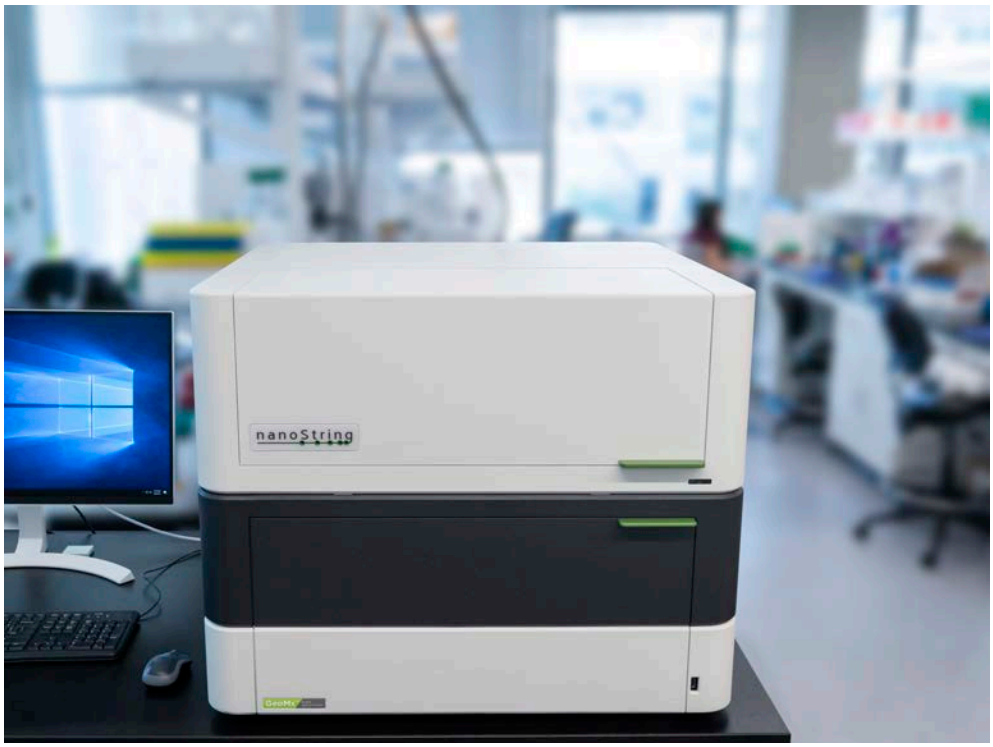


Figure 1: GeoMx DSP instrument

The GeoMx Digital Spatial Profiler (see Figure 1) is a benchtop instrument designed to spatially resolve and collect genomic material from distinct, user-defined, regions of interest. This **GeoMx DSP Instrument manual** is concerned exclusively with the operation of the GeoMx Digital Spatial Profiler. It is important that you read and comprehend the provided information. Please contact geomxsupport@nanostring.com to address any questions or concerns.

Separate topics and manuals provide information on **slide preparation**, the transition of samples and data from the GeoMx DSP instrument to an **nCounter** or **NGS platform**, and the final **data analysis**.

Figure 2: Help icon in Control Center

They exist as individual PDF manuals and as one collective **GeoMx DSP Online User Manual**. Click on the help link in the DSP Control Center (see Figure 2) or visit https://www.nanostring.com/geomx-online-user-manual to access the home page.

INSTRUMENT COMPONENTS

Figure 3: GeoMx DSP instrument exterior diagram

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

The front of the instrument boasts a USB port (USB-A 3.0) and a barcode scanner **(see Figure 3)**.

The main (upper) door opens to the GeoMx DSP instrument stage **(see Figure 4)**. The reagent bay (lower) door provides access to the buffer bottles and waste container.

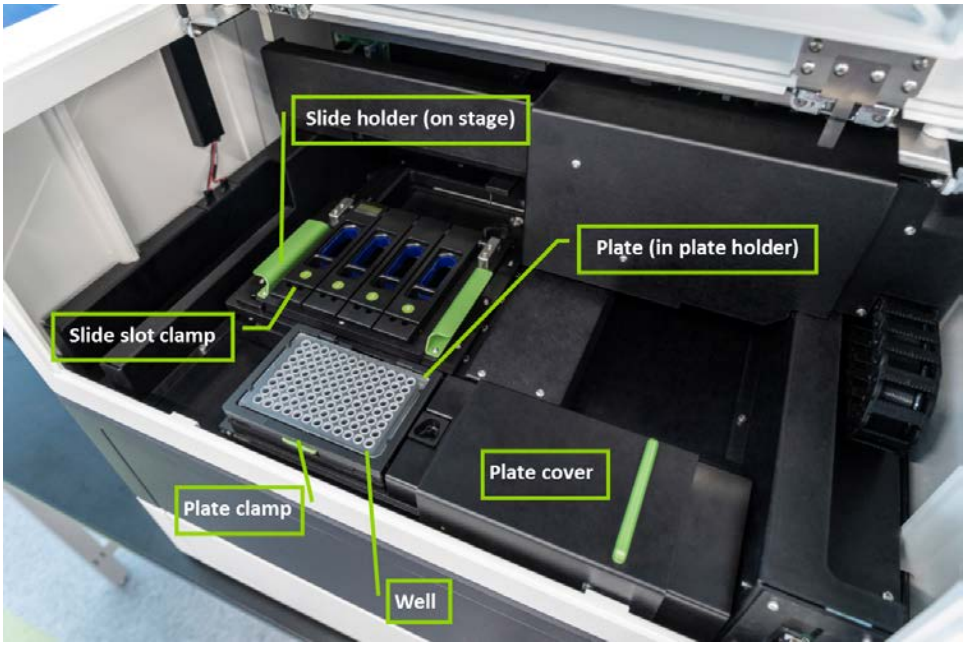


Figure 4: GeoMx DSP instrument interior diagram

Open the main (upper) door to access the GeoMx DSP instrument stage. Here, you'll load the collection plate into the plate holder and secure it with the plate clamp. If your collection plate is loaded before your slides, the system will prompt you to slide the plate cover over the collection plate until loading is complete. Slides are loaded into slots on the slide holder in a designated lab space, secured with the slide slot clamps, and covered with buffer. The slide holder is then fitted onto the stage of the instrument.

Figure 5: GeoMx DSP instrument reagent bay diagram

The GeoMx DSP instrument requires the following reagent bottles in the reagent bay (see Figure 5):

- **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:** Ensure the instrument is in an idle state before changing reagents (see Idle State Pages). In addition, DO NOT top off any reagents. Rather, replace entire bottles through the wizard when needed.

For information on loading, removing, and replenishing reagent containers, see GeoMx DSP Instrument Reagents on page 100.

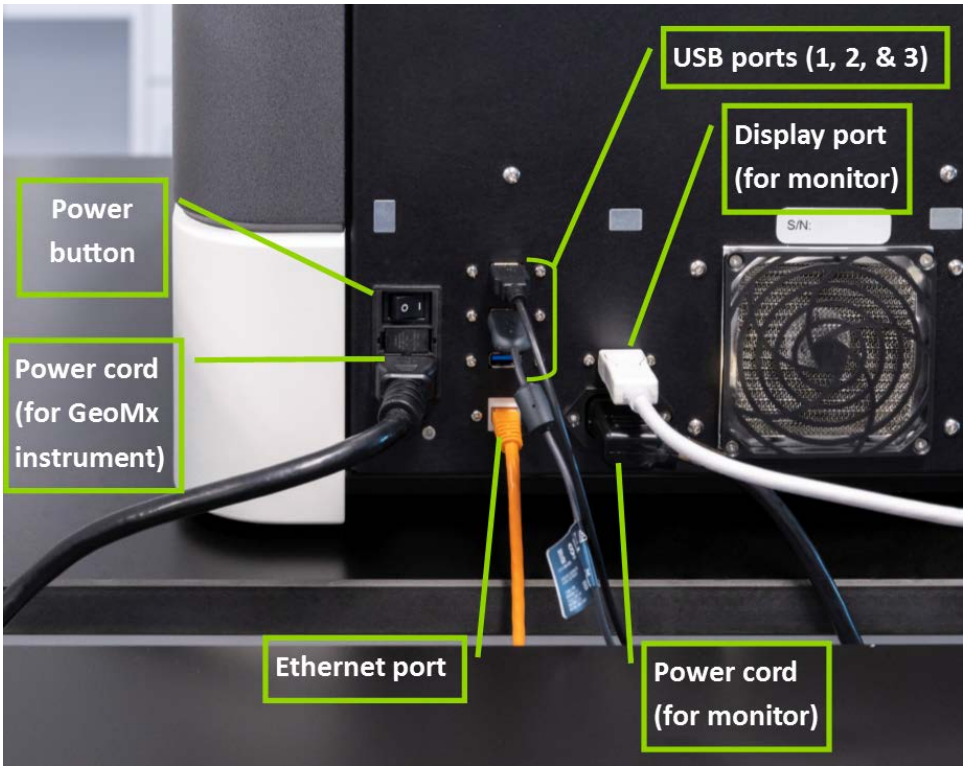


Figure 6: GeoMx DSP instrument backside diagram

The backside of the GeoMx DSP instrument **(see Figure 6)** offers ports for the following:

- 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. The necessary cables and a waste bottle are included with each instrument. Never dispose of the waste bottle. All other consumables and reagents may be purchased separately. Instrument specifications are listed below **(see Table 1)**
.

Table 1: GeoMx instrument specifications

Slides per Run	1 - 4 (1x3") slides per run
Weight	210 lbs
Dimensions (W x D x H)	30 in (760 mm) W x 29 in (733 mm) D x 24 in (610 mm) H
Power Requirements	100 - 240 VAC
Fuse	50/60 Hz 4A
Temperature	18–28°C
Humidity	30–80% relative humidity (non-condensing)

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 movement of the instrument. See **Safety Information (English) on page 14**.
- 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" for ventilation.
- Ensure adequate clearance on the front of the instrument to open 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 which require additional instruction in this manual, however, some general safety guidelines are provided in **Safety Information (English) on page 14** and in **Disposal of Electronic Equipment on page 13**.

PRODUCT USE LIMITATIONS

The GeoMx DSP system is intended for research use only.

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 (WEE 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 dealer or local NanoString office for the proper decontamination information and take-back program, which will facilitate the proper collection, treatment, recovery, recycling, and safe disposal of the device.

SAFETY INFORMATION (ENGLISH)

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

If the GeoMx 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. When lifting a load heavier 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).

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. Properly shutdown, then disconnect the instrument from the power source before attempting to replace the fan filter.

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. The 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

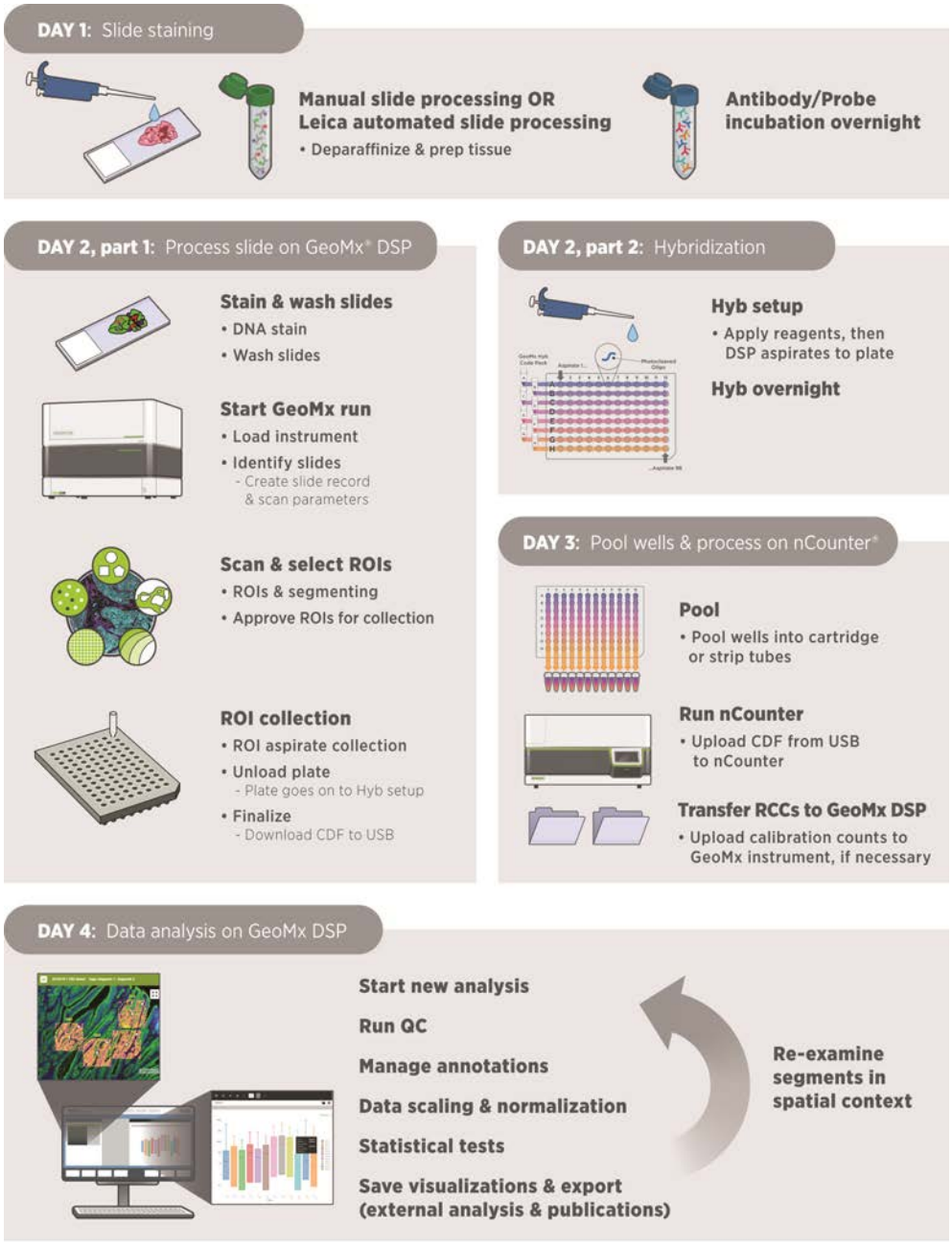


Figure 7: GeoMx DSP overall workflow

The GeoMx® Digital Spatial Profiling (DSP) technology is a novel platform developed by NanoString. This product relies upon antibody or RNA probes coupled to photocleavable oligonucleotide tags. After the hybridization of probes to slide-mounted tissue sections, the oligonucleotide tags are released from discrete regions of the tissue via UV exposure. Released

tags are quantitated in an nCounter® or Illumina NGS assay, and counts are mapped back to tissue location, yielding a spatially-resolved digital profile of analyte abundance. The GeoMx DSP workflow describes the process of accomplishing these steps **(see Figure 7)**.

- **Slide Staining** is mostly performed on Day 1. During this phase, you will prepare slides and hybridize biological targets with UV-cleavable biological probes.
- On Day 2, you will label cell types of interest with fluorescent morphology markers, then go into the next phase, **Process Slide on GeoMx DSP**. During this phase, you will load your prepared slides onto the GeoMx DSP instrument, enter identifying information for them, scan them to create fluorescent images, select regions of interest (ROIs), and then collect UV-cleaved oligos from these ROIs into the wells of a collection plate.
- If you are running your readout on an **nCounter** platform, you will transfer the contents of the DSP collection plate to a hybridization plate after the GeoMx DSP run on Day 2 and set up the overnight **Hybridization** step with GeoMx Hyb Code reagents.
- On Day 3, you will **pool wells** and **process on an nCounter platform**. Compressed data files (RCCs) describing these counts can then be transferred back to the GeoMx DSP instrument for **Data Analysis** on Day 4.

GeoMx DSP User Manuals and other user documentation

- All of the GeoMx DSP user documentation exists in the **GeoMx DSP Online User Manual**, accessible from the help icon on the **GeoMx DSP Control Center** and online at https://www.nanostring.com/geomx-online-user-manual.
- PDF versions of GeoMx DSP documentation are also available for both nCounter and NGS readouts. The **Slide Prep**, **Instrument**, **Readout**, **GeoMx NGS Pipeline (DND)** (for NGS only), and **Data Analysis** user manuals are available for download from the **GeoMx DSP Online User Manual** (see above).
- nCounter MAX/FLEX and SPRINT documentation can be found in their respective manuals: nCounter Analysis System MAX/FLEX (https://www.nanostring.com/download_file/view/256) and nCounter SPRINT (https://www.nanostring.com/download_file/view/254).

GeoMx DSP Run Introduction

The GeoMx DSP Control Center is the main interface of the GeoMx DSP software. It allows you to control all instrument tasks including creating slide records (see **Creating Slide Records on page 67**), managing users (see **Administration on page 76**), accessing Data Analysis (see the **GeoMx DSP Data Analysis User Manual**), and starting a run (**Data Collection**; see below). Here, we introduce the Data Collection process and user interface.

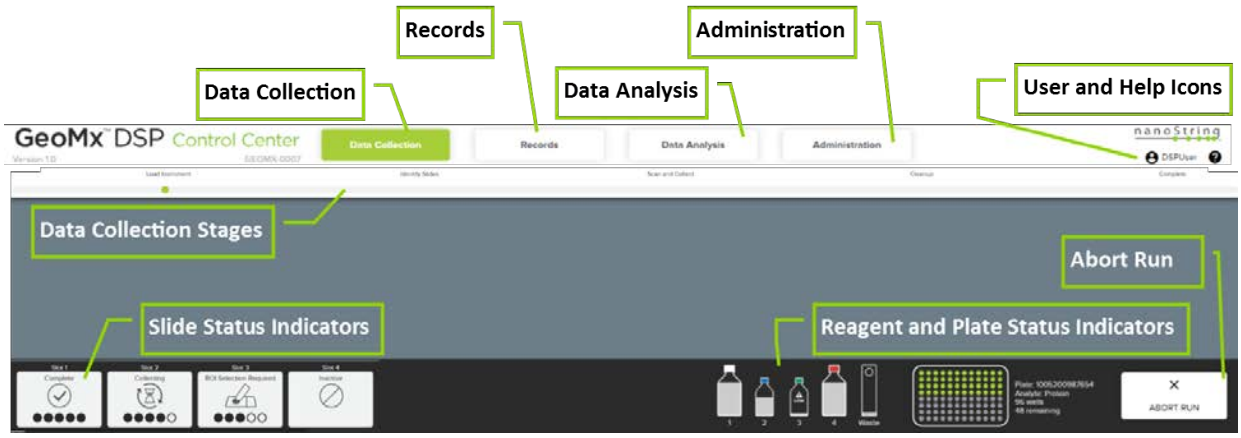


Figure 8: GeoMx DSP Control Center diagram

By clicking the **Data Collection** button in the GeoMx DSP Control Center header **(see Figure 8)**, you can begin the run (starting with loading the instrument) or upload nCounter counts (including calibration data). 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 is available to administrative users. The **Data Collection Stages**, depicted just below the header when **Data Collection** is selected, provide you with a visual orientation of where your run is in the process. **Slide** and **Plate Status** indicators in the footer indicate the current state of the slides and plate, respectively. **Reagent Status** indicators inform on 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 Quick Guide

Download the GeoMx DSP Instrument Quick Guide from https://www.nanostring.com/geomx-online-user-manual.

GeoMx DSP Instrument Channel Details

The wavelengths and excitation and emission information are listed below (see Table 2) for each of the GeoMx DSP channels.

Table 2: GeoMx channel information

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

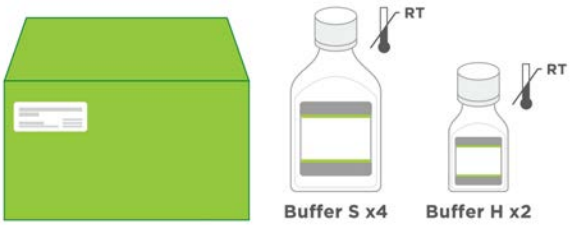
Loading the Instrument

Loading the GeoMx DSP instrument is a workflow mostly guided by the GeoMx DSP system software. You may choose to perform some of the optional pre-work steps, which include creating slide records and assigning scan parameters. See **Creating Slide Records on page 67**.

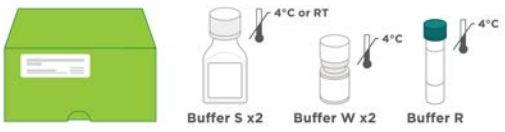
REAGENTS AND CONSUMABLES NEEDED

In addition to the reagents in the kits listed below, you will need lint-free wipes, **dH_20**, 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)

1 Start the run

Start the run by hovering over the **Data Collection** button (see Figure 9) in the DSP Control Center and selecting **New/Continue Run**.

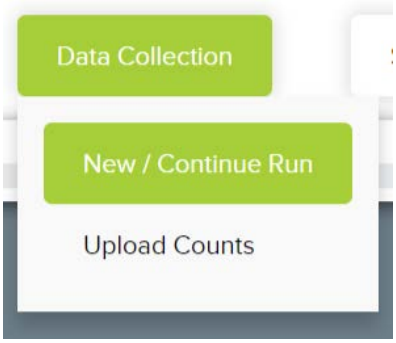


Figure 9: New/Continue Run button

If a Data Collection run is in progress, you will be taken to the current step in the run.

2 Load the slides into the slide holder

After DSP slides have been prepared, they should be immediately loaded into a GeoMx DSP slide holder (see Figure 10) and covered in buffer in a designated laboratory space. Alternatively, if using the safe storage guidelines (see the **GeoMx DSP Slide Preparation User Manual**), slides may be stored for up to 6 hrs or longer after preparation. Storing slides for longer amounts of time or deviating from the safe storage guidelines may result in reductions in data quality.

Figure 10: Loading the slide holder

LOADING THE SLIDES

1. Obtain a clean slide holder 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** for cleaning guidelines.

   IMPORTANT: Prior to loading slides in the tray, ensure that the spring-loaded pins in the upper left corner of each slide holder are in the retracted position. If they have become stuck due to salt buildup, they must be manually pushed into the retracted position. Failure to do this can result in breaking the slide. Failure to clear material can also affect gasket sealing, causing instrument damage.

   IMPORTANT: Slide labels must be carefully applied to one end of the slide and must not be positioned beyond the frosted portion of the slide or under the gasket of the slide holder. Avoid using dark matted or colored labels since the imaging system needs to automatically detect label areas.

2. Inspect your slide for debris and residue.
   - Clean the bottom of your slide with a lint-free wipe and **70% ethanol**.
   - Inspect your slide to ensure there is no residual material from the hydrophobic pen used during slide preparation. See the **GeoMx DSP Slide Preparation User Manual**.

3. Place your slide in the slide holder, face up, label toward (closest to) you **(see Figure 11)**. Ensure that the slide is evenly seated in the slide holder slot.

Figure 11: Loading the slide holder

4. Lower the slide holder clamp (see Figure 12).
   - The slide label should be visible in the rectangular window above the green slide slot number.
   - The tissue should be visible in the elongated window.
   - Clean the bottom of the slide again, if needed, with a lint-free wipe and **70% ethanol**.

Figure 12: Lowering the slide holder clamps

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

5. Add **6 mL Buffer S** to each tissue section (see Figure 13). This reagent is included in the slide preparation kit. Do NOT remove Buffer S from the GeoMx instrument reagent bay. The gasket on the slide holder clamp should contain the buffer so there is no leaking into the instrument.

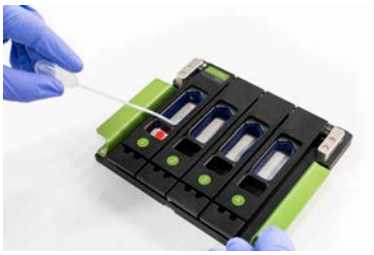


Figure 13: Adding buffer

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 slots in which Slide IDs are loaded:

3 Set up the GeoMx DSP instrument

Follow the prompts on the GeoMx DSP screen. For more information on a particular screen, see below. Otherwise, proceed to **Identify Slides on page 32**.

Depending on whether you are using a preloaded collection plate or a new one, you may see the following screens:

REPLACE PLATE?

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

Select **Yes** to replace the collection plate; you may need to finalize the plate (see **Finalizing the collection plate on page 65**). Select **No** to use the collection plate currently loaded on the GeoMx DSP instrument.

- **Previously completely used collection plate:** if the plate in the previous run was completely filled, you will be prompted to use a different plate.
- **Previously partially used collection plate:** if the plate in the previous run was only partially filled, you may choose to use the remaining wells for the present run. Collection will be set to use the next available well on the plate.

Select **Next.**

OPEN/CLOSE THE DOOR

Open the instrument door when prompted. Once the door is open (see Figure 14), the system will display the next step.

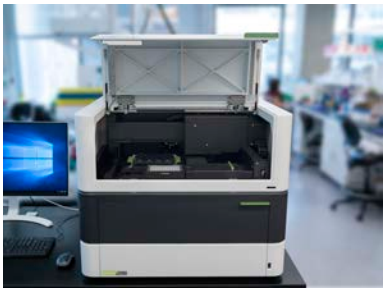


Figure 14: Open the GeoMx instrument door when prompted.

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

Close the instrument door when prompted (see Figure 15).

The system will determine that the plate cover is out of the way of the plate and then lock the door.

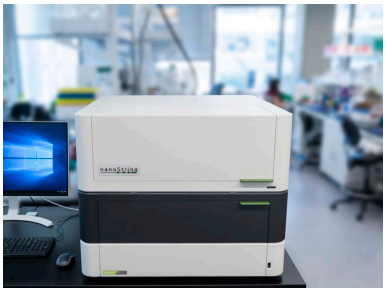


Figure 15: Close the GeoMx instrument door when prompted.

INSERT THE SLIDE HOLDER

For information on loading the slides into the slide holder, see Loading the Slides on page 25.

- Load the slide holder onto the stage of the instrument (see Figure 16) when prompted.
- Inspect visually and by touching to ensure that the slide holder fits evenly in the frame of the stage.
- Pointed ends of the slide slots should be pointing to the back of the instrument.

Figure 16: 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 64.

IDENTIFY PLATE

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

Scan the plate's barcode by holding it at a 45° upward-facing angle 4 - 6 inches from the barcode scanner on the front of the instrument. The barcode will auto-fill when read. Alternatively, you may enter the barcode manually.

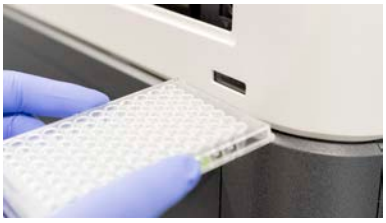


Figure 17: Scanning the plate barcode

- **New collection plate:** If the barcode has not been previously read by the instrument, it is assumed to be new. Aspirate collection will be set to the first well.
- **Previously completely-used collection 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.
- **Previously partially-used collection plate:** if the plate's barcode is recognized and it was previously partially-filled, collection will be set to use the next available well on the plate.

> **IMPORTANT: For nCounter readout, the row into which aspirates are collected must match the HybCode** used later in the workflow; a hybcode must always be loaded into the row matching its name (i.e., Hybcode A should always be loaded into row A and interact with aspirates from row A). If you collect the aspirate into row C, you must use HybCode C for the nCounter readout.

Only one analyte type is allowed per collection plate.

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

Click **Next**.

COVER/UNCOVER THE COLLECTION PLATE

This instruction will appear if your collection plate is loaded before your slides. Covering the collection plate (see Figure 18) as you load the slide holder onto the stage prevents the transfer of potential contaminants from the slides to the collection plate wells. Once the plate cover has been moved over the plate, the system will display the next step.

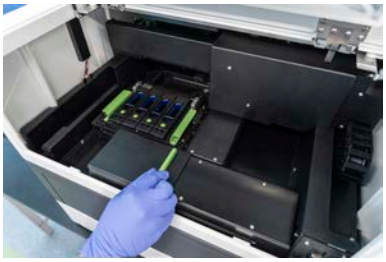


Figure 18: Cover the collection plate when prompted

Once the slides and collection plate have been loaded, you will be prompted to uncover the collection plate. Once the plate cover has been moved out of the way of the plate (see Figure 19), the system will display the next step.

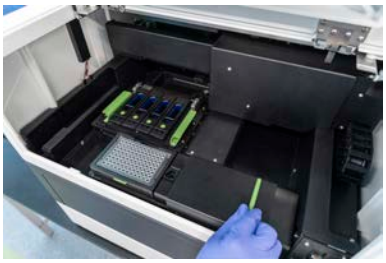


Figure 19: Uncover the collection plate when prompted

INSERT THE COLLECTION PLATE

To insert a plate:

1. Open the plate holder clamp.
2. Insert the collection plate onto the plate holder (see Figure 20).

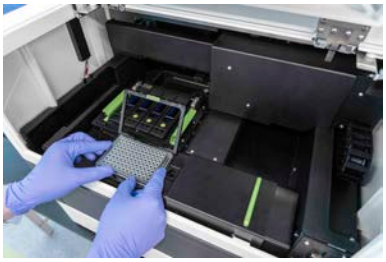


Figure 20: Insert the collection plate onto the plate holder when prompted

3. Fasten the clamp (see Figure 21).
4. Once the clamp is fastened, the system will display the next step.

For information on removing a plate, see Finalizing the collection plate on page 65.

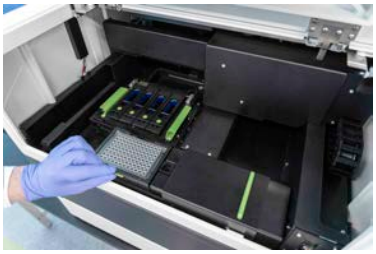


Figure 21: Fasten the clamp on the collection plate

The system will detect the necessary components for the run. It will then proceed to Identify Slides on page 32.

Identify Slides

Once loading the instrument is complete and the necessary components for the run have been detected, the system will commence **Slide Identification** by taking a picture of each slide **(see Figure 22)**.

Figure 22: Identify Slides stage in GeoMx DSP run workflow

4 Identify Slides

VERIFY OR COMPLETE THE FIELDS UNDERNEATH EACH SLIDE IMAGE

You may link slides to any slide records and scan parameters created earlier **(see Figure 23)**.

- **Slide Record**: This record contains the metadata for the slide. You may click the **Create** button or the **Look Up** button (see **Slide records on page 34**).
- **Barcode**: This field auto-populates with the slide record barcode (if entered) when linked to a slide record.
- **Scan**: This contains the parameters for the scan.
  - If you established scan parameters in advance, they will appear in the drop-down menu here; select a set of scan parameters here.
  - If not, select **Create New Scan** from the drop-down (see **Scan Parameters on page 35**).

Figure 23: Slide slot view during Identify Slides stage

- **Exclude this slot:** Check this box to exclude the slide from further processing.

SLIDE RECORDS

If the slide record field is blank, you will need to either look up a previously-created slide record or create a new one at this time.

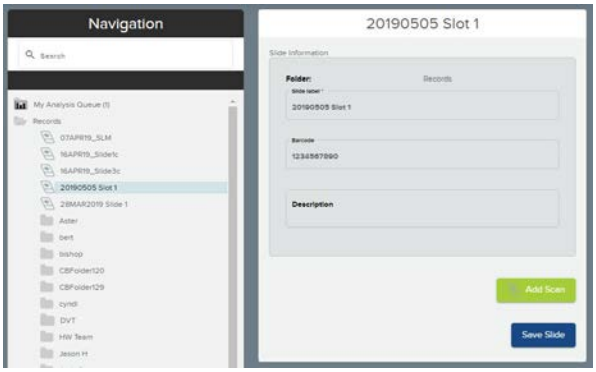


Figure 24: Navigating to a slide record

Look up:

- From the **Identify Slides** window, select **Look up slide record**.
- 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 **(see Figure 24)**.
- Click the **Select Slide** button.

Create:

- From the **Identify Slides** window, select **Create slide record**.
- Open or create a new parent folder from the **Navigation** window.
- The **Creating new slide record** window will appear to the right of the Navigation window. Fill in:
  - **Slide Label**: the unique identifier which will show in the Navigation window
  - **Barcode** (optional)
  - **Description** (optional)
- Click the **Save Slide** button.

SCAN PARAMETERS

Scan parameters contain the settings for the scan **(see Figure 25)**. Review the options in the Scan Parameters field at the bottom of the Slide ID window. You may select one of the sets in the drop-down field or create a new set.

1. **Enter a Name** for this scan. This should be a unique identifier.

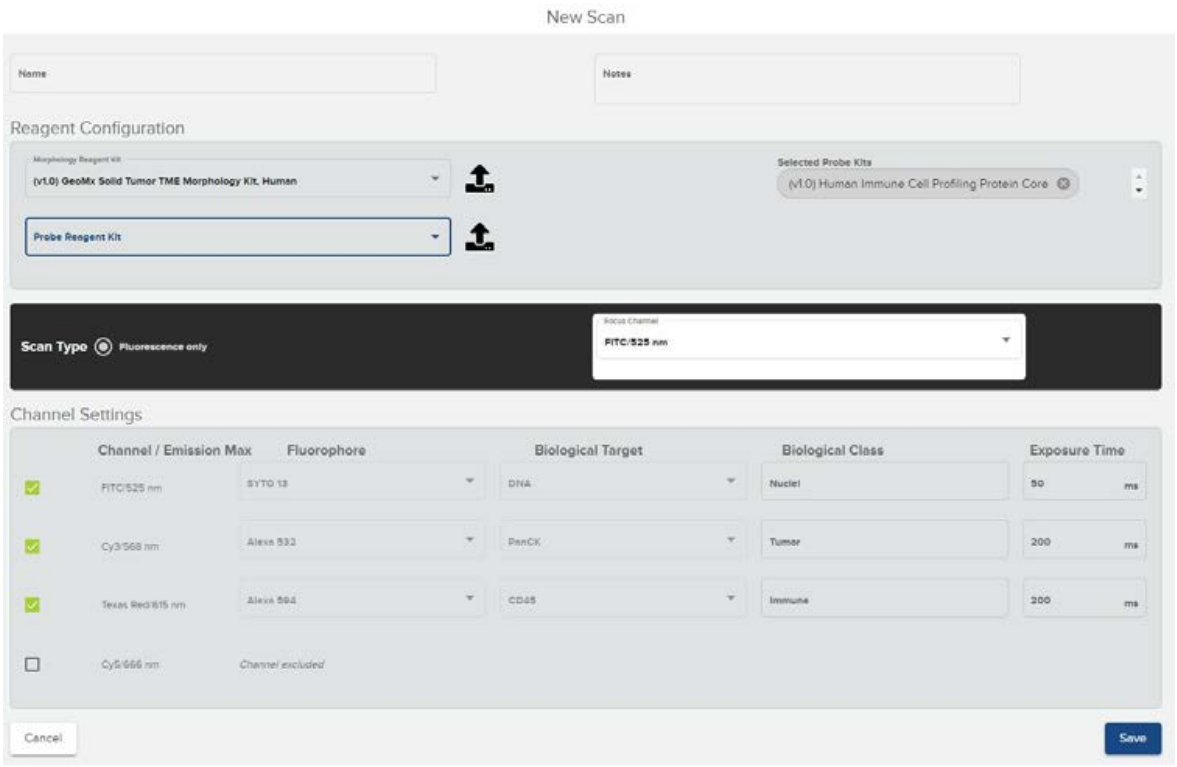


Figure 25: Scan Parameters

2. **Upload/Select Reagent Configuration files.**

   GeoMx DSP configuration files associate RNA and Protein assay targets with GeoMx readout barcodes. Morphology Kit configuration files are used to auto populate key target information for ROI selection in the GeoMx DSP Software. Find the files for the kits used on your slide at www.nanostring.com/dspconfigfiles. Save these files to a USB drive.

   - Select the **Morphology Reagent Kit** used in preparing the slide from the drop-down. If not already loaded, select the **upload** button to the right of this field to upload the respective configuration file from your USB drive (see above). See **Example Scan Parameters on page 72** for GeoMx Solid Tumor TME and Melanoma Morphology parameters.
   - Select any probe kits used in preparing the slide from the **Probe Reagent Kit** drop-down. Select the **upload** button to upload configuration files from your USB drive, if needed. Any selected probe kits will be listed to the right of the upload buttons. The Core Kit must be selected before the compatible Module Kits will be listed.

   IMPORTANT: ***Select the file that matches the name, lot number, and Probe R space of the GeoMx Assays used.*** Ensure you have uploaded the correct configuration files for the core and each module used during slide prep. 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).**
   - Proceeding through aspirate collection with **an incorrect *.pkc* file** may result in a **permanent misassociation of probes** and invalid data displayed in Data Analysis.
   - For any Substitute Probe R (**Sub Probe R**) used in **Protein nCounter** slide preparation, a **Sub Probe R configuration file** must be used. See Appendix V of the **GeoMx-nCounter Slide Preparation User Manual** for more information on using a Sub Probe R.
   - If spiking in **custom targets** to an existing GeoMx assay, NanoString's bioinformatics department will provide a *.pkc* file to be uploaded in the Probe Reagent Kit field in addition to the GeoMx assay *.pkc*.
   - If adding **custom RNA targets to an nCounter** assay, only one custom RNA module should be used per scan.
   - If a protein module added to the list shares the same reporter space as another module on the list, a warning will appear; carrying forward with this scenario poses the risk of losing data from both modules. Remove one of the conflicting modules.
   - If you do not see the file you need, contact geomxsupport@nanostring.com for assistance.

Configuration files contain probe group designations which will materialize in the Data Analysis stage.

3. Select the appropriate **Scan Type** and **Focus Channel** for your slide.
   - The scan type option will be set to **Fluorescence** only; the **Brightfield only** and **Fluorescence and Brightfield** options are inactive at this time.
   - Focus channel options (for fluorescence): **FITC / 525 nm**, **Cy3 / 568 nm**, **Texas Red / 615 nm**, and **Cy5 / 666 nm**. The **nuclear dye** (default in the **FITC/525 nm** channel) is typically used as a focus channel.
4. Verify the **Channel Settings** fields.
   - Ensure all channels you wish to use in the scan have been checked.
   - Adjust the value in the **Exposure Time** field, if desired.
   - Adjust the name in the **Biological Class** field, if desired.
5. Fill in the custom morphology field (if applicable) to populate custom fluorescence exposure settings.
6. Select **Save**.

Select **Next**. The system will proceed to the **Review Tissue Detection** window. See **Scan on page 38**.

Scan

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

5 Define Scan Area

The scan area result will be displayed (see Figure 26). Use the tools in this window to select *only* the tissue on the slide, minimize scan time, and avoid unnecessarily consuming disk space. 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 bounding box using the **X- and Y- sliders** to define the area you would like to analyze.

> **IMPORTANT:** If you have multiple sections on your slide and only wish to scan and collect data from a particular one, use the bounding box to limit the area considered for scanning.

- Use the **Minimum Tissue** slider to filter particles from the image. Exercise moderation when using this slider, as sliding it too far may cause small areas of tissue to be excluded from the image.
- Use the **Sensitivity** sliders to establish the tissue boundaries. Pay careful attention in the case of small or fragmented tissues.

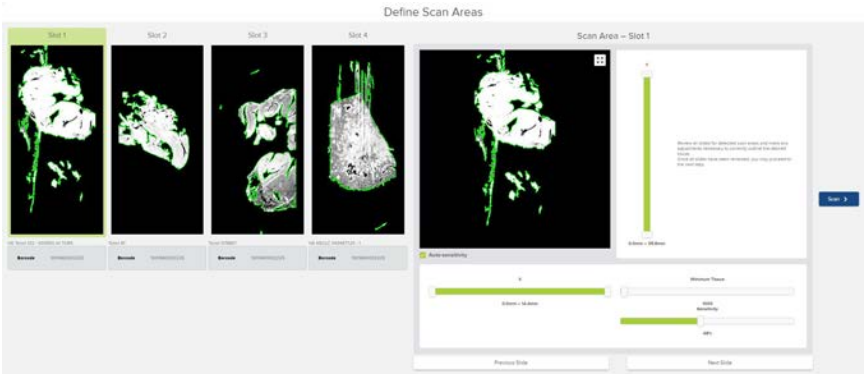


Figure 26: Scan area definition adjustment

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 commences.

Once through all slides, select the **Scan** button.

6 Scan

The system will commence taking a high-magnification scan of each slide, using the tissue area just defined and using the scan parameters specified in the scan record (see Figure 27).

- Once the entire 20x scan has been completed for a slide, you may zoom in, zoom out, pan the image, 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.

See ROI Selection & Collection on page 41.

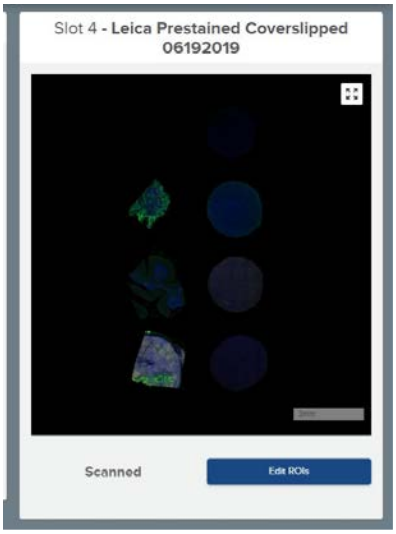


Figure 27: 20x scan and Edit ROIs button

ROI Selection & Collection

The next stage of the GeoMx DSP run is ROI Section, followed by Collection.

The Scan Workspace (see Figure 28) allows you to position regions of interest (ROIs) on your scan. If desired, you can also segment them within the Scan Workspace or in an external program. Once you have completed this process, the GeoMx DSP instrument then exposes each segment of each ROI to UV light and aspirates material from the solution into a well on the collection plate.

Maximum ROI size is 660 µm x 785 µm. Additional guidance for protein and RNA is provided below.

7 Select ROIs

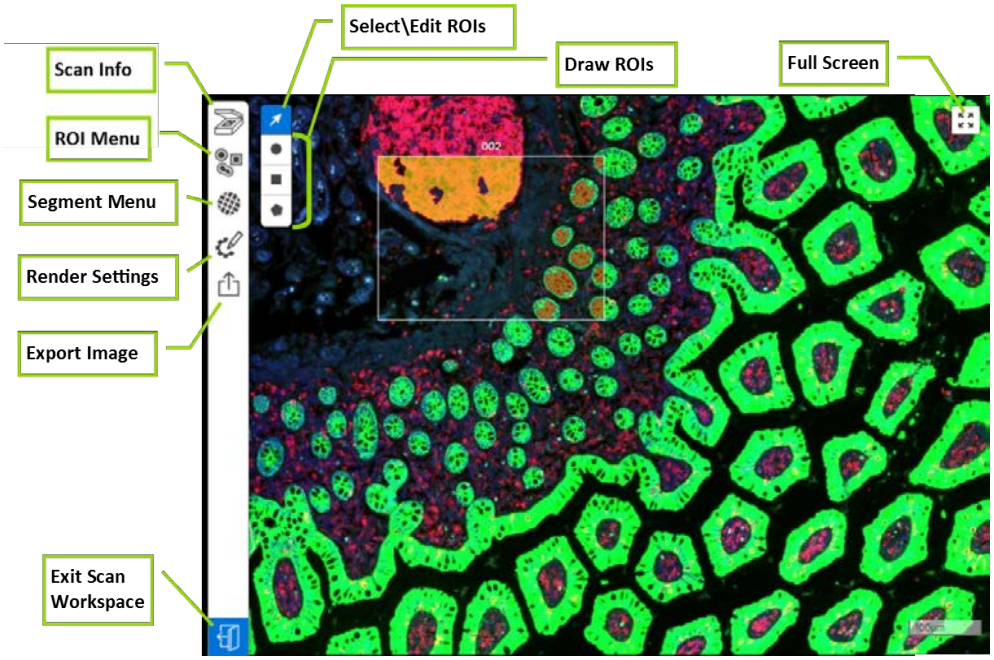


Figure 28: Scan workspace diagram

OPEN THE SCAN WORKSPACE

There are two ways to access the Scan Workspace:

- During the DSP run, use the **Edit ROIs** button (see Figure 29), which will become active underneath a slide's image after the 20x scan has completed.

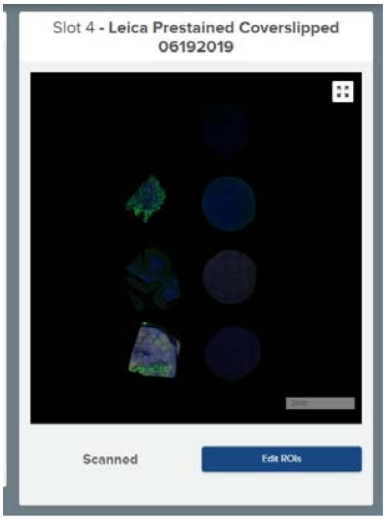


Figure 29: Control Center single slot view of Edit ROIs button activated after scanning

- 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 your scan card, select the **Scan Workspace** link (see Figure 30).

Figure 30: Scan card with Scan Workspace link

INSPECT THE IMAGE

- Ensure that the scan quality is acceptable for designation of ROIs and segments. Acquiring from out of focus images should be avoided, as it may cause instrument failure.
- Note that red blood cell autofluorescence is very common in FFPE tissues; take care to 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 at right for keyboard commands for scrolling and zooming.

Keyboard Shortcuts

- (zoom out) or **_** (super-zoom out)

= (zoom in) or **+** (super-zoom in)

← (pan image right) or **shift** ← (super-pan image right)

↑ (pan image up) or **shift**↑ (super-pan image up)

→ (pan image left) or **shift** → (super-pan image left)

↓ (pan image down) or **shift**↓ (super-pan image down)

Number keys **1**, **2**, **3**, **4** select the **pointer**, **circle**, **square**, **polygon** ROI tools, respectively.

- Open **Render Settings** (see Figure 31). Here, you may:
  - Change the colors to represent the different channels on the scan.
  - Adjust the intensity of each channel, either with the slide bar or the editable **Min** and **Max** boxes.
  - Use the **Undo**, **Redo**, or **Revert** buttons at the bottom of the Render Settings window, if needed.

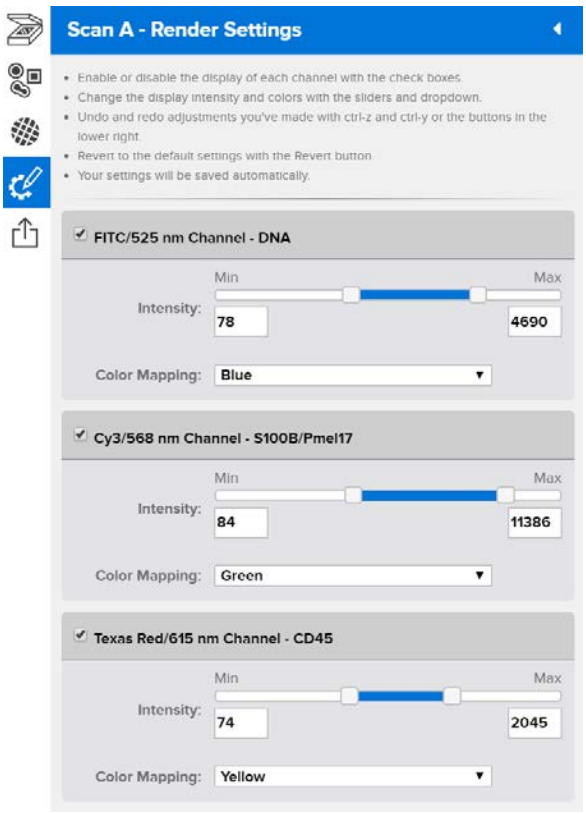


Figure 31: Scan Workspace Render Settings menu

DRAW ROIS

Choose from geometric shapes (circle or rectangle) and custom polygon.

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

Decide on your strategy before commencing (i.e., Geometric ROIs only, Segmented (tumor/stroma) ROIs only, etc.). If collecting from a combination, establish the segmented ROIs *first*, then place the geometric ROIs.

STANDARD GEOMETRIC SHAPES

1. **Select a shape button (circle or rectangle)** from the Scan Workspace (see Figure 32).
2. **Click in the corner** of the area you would like to designate as an ROI and drag your mouse; watch the ROI expand in the shape you specified.

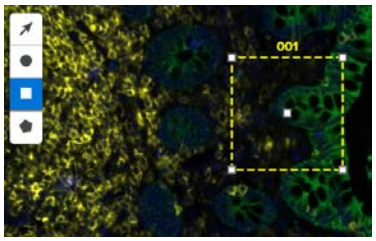


Figure 32: Scan Workspace square ROI button

For protein analysis, the diameter of a circular ROI or side of a rectangular ROI should be ~50 - 400 µm (~200 µm is ideal). ROIs that will be segmented may be slightly larger, ~100 - 400 µm (~300 µm is ideal if segmented). For RNA analysis, ROI diameter should be >200 µm (>400 µm if segmented). Open the ROIs List to check the dimensions of established ROIs.

3. **Release the mouse click** to complete the ROI. This most-recently applied ROI will now have a number designation.

You can copy and paste an existing ROI or multiple ROIs by holding down the control button (Ctrl) on the keyboard and clicking and dragging the center point of an existing ROI. You should see an identical ROI(s) appear. Release the click to place this ROI(s).

Shift-drawing a rectangle with the pointer tool zooms in on that rectangle.

4. Once you have placed the ROI, you may adjust it.
   - Click and drag the center point of the shape to move the shape.
   - Click and drag a white perimeter point to re-size the shape.
   - To segment ROIs, see **Segment ROIs (if applicable) on page 49**.

- 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).

5. Open the **ROIs List** (see Figure 33) to:

- Use the **Export all** button to export all ROIs in high-resolution tiled .tiff format for custom segmenting.
- Change the ROI numbering to any unique alpha-numeric choice. If doing this, 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 ' \ / * : < > | " '.? in the ROI or segment names.
- Manually change the dimensions or location of an ROI.
- Add a **Comment** to the ROI.
- **Export** the individual ROI in high-resolution tiled .tiff format for custom segmenting or **Import** a segment mask (see Using an External Program for Segmenting on page 56) .
- **Delete** an ROI.

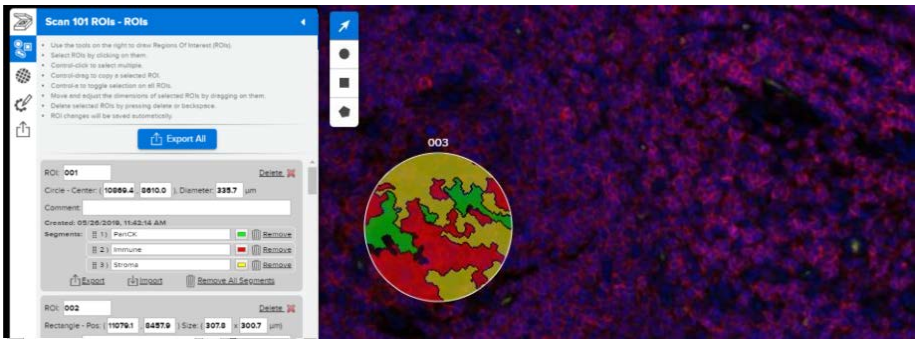


Figure 33: Scan Workspace ROIs List with segments

CUSTOM POLYGON

1. **Select the polygon button** from the Scan Workspace (see Figure 34).
2. **Single-click where you would like one vertex** of the polygon; drag your mouse and watch a line grow from this vertex.
3. **Single-click to create more vertices** and watch the ROI form.

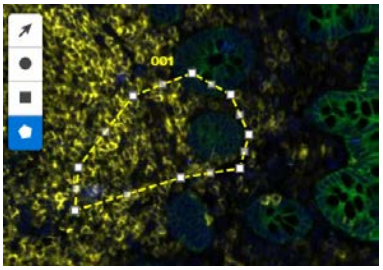


Figure 34: Scan Workspace polygon button

4. **Click back on the original vertex to complete the ROI.** This most recently applied ROI will now have a number designation.

> For protein analysis, the area of a polygon ROI should be between ~2,500 - 40,000 µm^2. For RNA analysis, the area of a polygon ROI should be >40,000 µm^2. Placing a 40,000 µm^2 square ROI over or near your polygon ROI may help estimate its size.

5. Once you have placed the ROI, you may adjust it.
   - Click and drag a vertex to adjust the polygon in shape and size.
   - Each side of a polygon has a center point. Simply click on a center point to convert it to a vertex.
   - Simply click on a vertex to convert it to a center point.
   - To segment ROIs, see Segment ROIs (if applicable) on page 49.
   - 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).

> You can copy and paste an existing ROI or multiple ROIs by holding down the control button (Ctrl) on the keyboard and clicking and dragging the center point of an existing ROI. You should see an identical ROI(s) appear. Release the click to place this ROI(s).

6. Open the **ROIs List**, if desired, to:
   - Add a **Comment** to the ROI.
   - Change the ROI numbering to any unique alpha-numeric choice. If doing this, 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 ‘ \ / * : < > | “ ‘.? in the ROI or segment names.

- **Delete** an ROI.

8 Segment ROIs (if applicable)

You can designate particular biological targets or combinations of biological targets as sub-regions, or segments, within your ROIs. To auto-segment all ROIs in the scan, use the Segments Panel within the Scan Workspace. See the **GeoMx DSP Segmentation Guidelines** at https://www.nanostring.com/geomx-online-user-manual.

Alternatively, you may export an ROI image for processing in an external program, such as ImageJ. See the **GeoMx DSP ImageJ Guidelines**, at https://www.nanostring.com/geomx-online-user-manual.

Establishing Thresholds for Segmenting ROIs in the Scan Workspace

This is an exercise to familiarize yourself with the channel thresholds and to establish the settings that may work well for your analysis. Choose the **Segments Panel** from the Scan Workspace sidebar.

1. Select **+Add Segment Definition** one time to establish a single definition rule. One gray Segment Definition box will appear **(see Figure 35)**.
2. **Choose one fluorescence channel** and set it to **+**. Leave the other channels set to ∅.

   Note: **Channel designations** determine whether each **fluorescence channel** (FITC, Cy3, Texas Red, Cy5) should be included (**+**), excluded (**-**), or ignored (∅) as part of the segment rule's definition.
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.

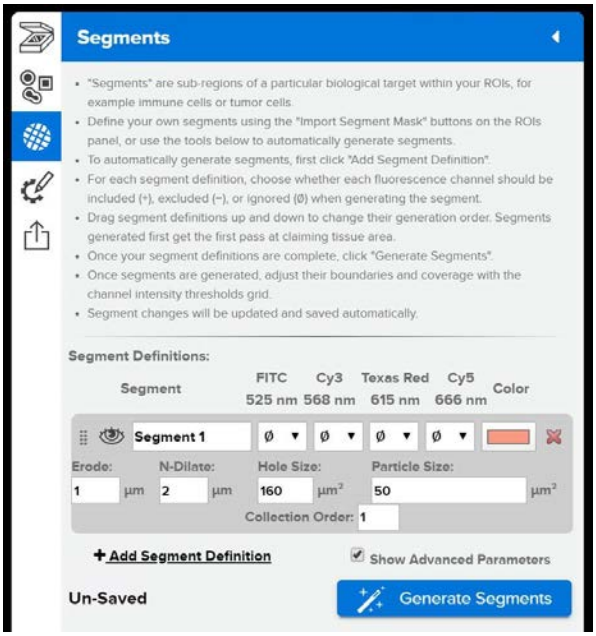


Figure 35: Scan Workspace segmentation menu

5. Adjust the value in the **Channel Thresholds** grid (see Figure 36), 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 **decrease the segment area**.
   - **Decreasing the Channel Thresholds** number will **increase the segment area**.
   - Adjust until the desired area is selected.
   - Make a note of this setting, although changes to values in this grid should be saved automatically.

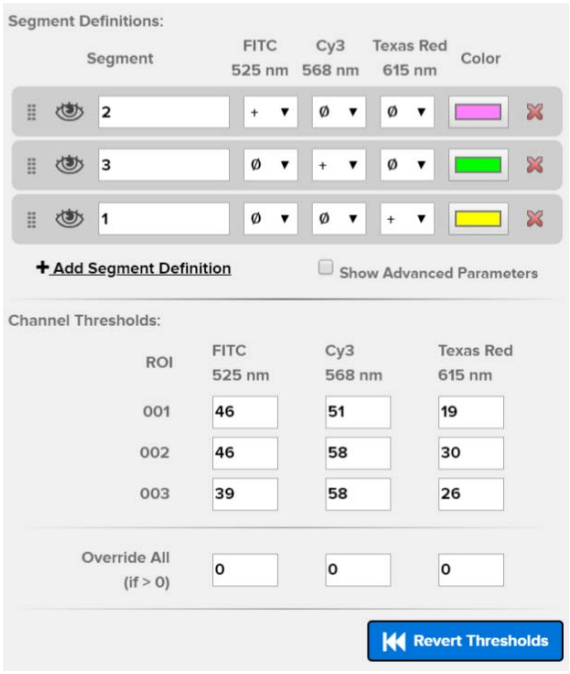


Figure 36: Segments with Channel Thresholds

6. You may delete this segment and **repeat steps 2-6 with a different channel designation** or simply change the settings on this segment definition rule and repeat steps 3-6 with a different channel designation.
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 51. Delete the segment definition box used for this thresholding exercise.

SEGMENTING ROIS IN THE SCAN WORKSPACE

1. Open the **Segments Panel** (see Figure 37), if it is not open already.

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.

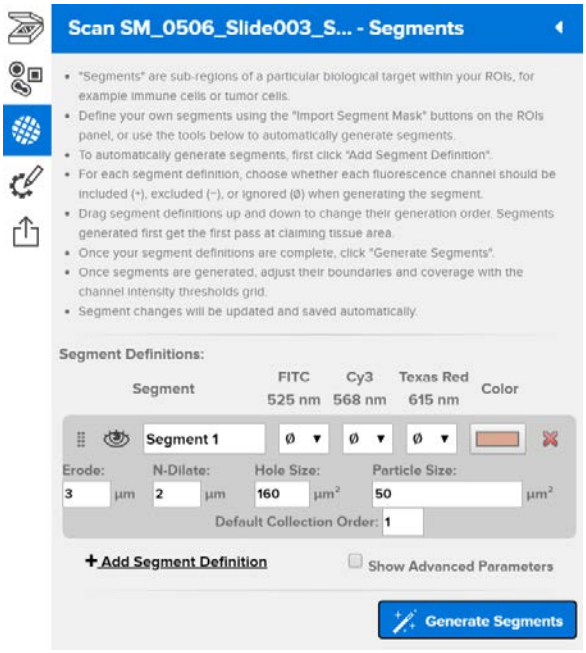


Figure 37: Scan Workspace segmentation workspace

3. For each Segment Definition box (see Figure 38), choose the **Segment Name** (to be used across all applicable ROIs). Do not use special characters like ' \ / * : < > | " '.? in the ROI or segment names. This segment name will materialize as a **tag** in the Data Analysis stage. See the **GeoMx DSP Data Analysis User Manual**.

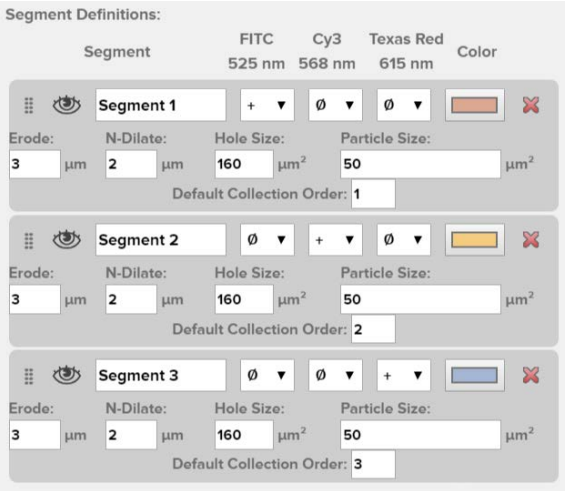


Figure 38: Segment definitions

4. **Channel designations**: For each segment definition box, choose whether each **fluorescence channel** (FITC, Cy3, Texas Red, Cy5) should be included (**+**), excluded (**-**), or ignored (∅) as part of the segment rule's definition **(see Figure 39)**.

   - Change the **segment color** used to illustrate the segment area on the scan, if desired. A default color will be present; click on the color box to choose a different one.
   - **Delete a segment**, if desired.

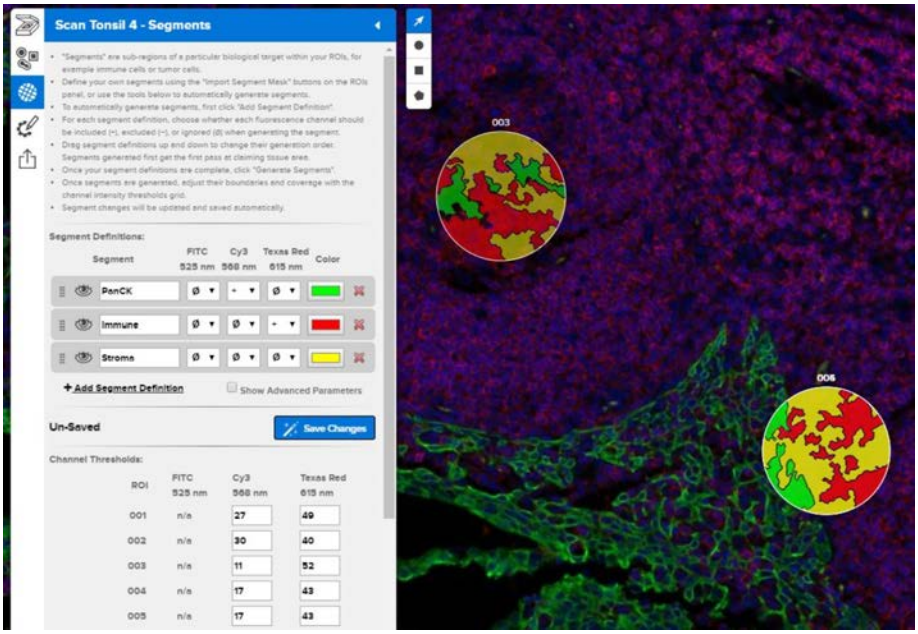


Figure 39: Segments generated in scan workspace

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 channel which is positive for DNA (usually FITC+), if used, should be set to be generated last.
   - Areas selected by 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** (see Figure 40).

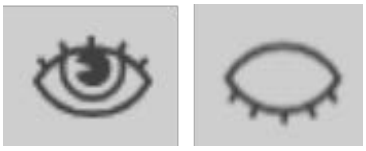


Figure 40: Visible segments and hidden segments clickable icon

7. Check the **Show Advanced Parameters** (see Figure 41). Here, you can adjust numerous parameters, including the order in which segments are generated and the order in which they are collected. See the **GeoMx DSP Segmentation Guidelines**, at https://www.nanostring.com/geomx-online-user-manual.

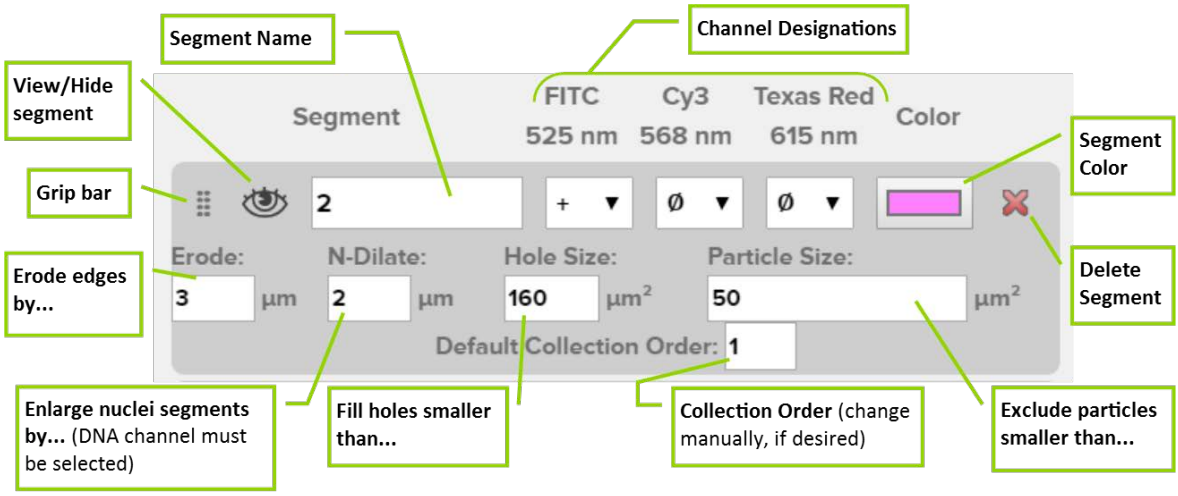
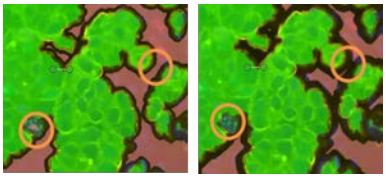
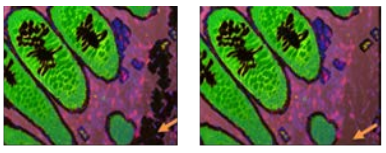


Figure 41: Segmentation advanced parameters

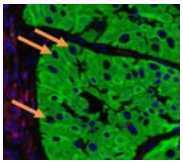
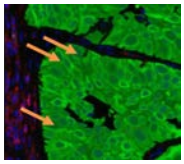
- **Erode:** The border width to be eroded from each segment; this effectively increases the boundary between segments. Increasing erode decreases segment size.



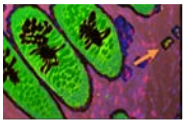
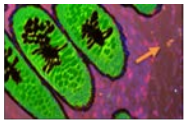
- **N-Dilate**: Adds segment area around detected nuclei. This setting is only applicable for nuclear- tagged segments.



- **Hole Size:** Holes smaller than this size in the detected segment area will be filled. 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 5. Segment generation order), 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 +) **(see Figure 42)**.

- **Increasing the Channel Thresholds** number will **decrease the segment area**.
- **Decreasing the Channel Thresholds** number will **increase the segment area**.
- 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.

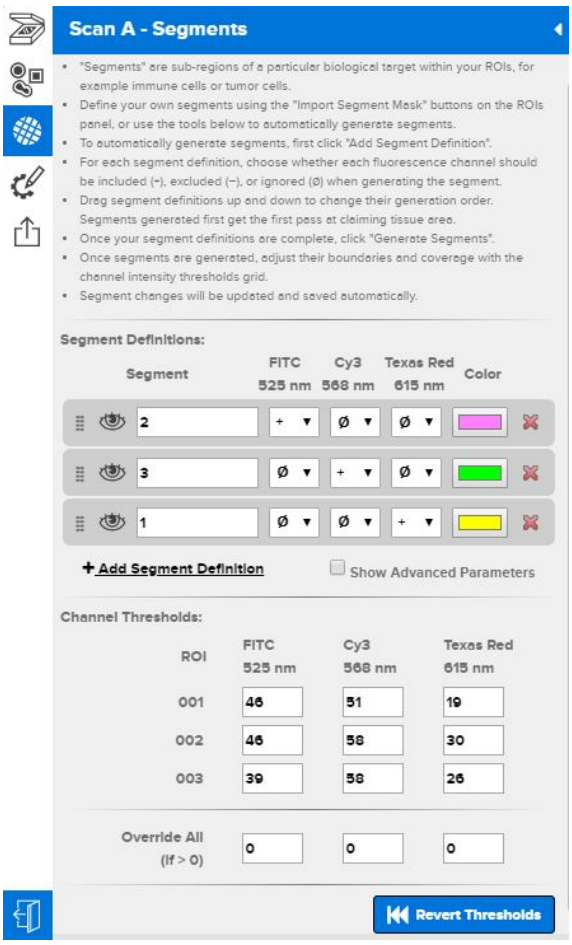


Figure 42: Segment definitions with channel thresholds

- If you make changes to the segment definitions, click the **Save Changes** button to ensure the changes are applied to the scan.

USING AN EXTERNAL PROGRAM FOR SEGMENTING

The GeoMx DSP system can export images to an external program (such as ImageJ) to create segments within an ROI. This is an optional process, available to users who require customization beyond that offered in the GeoMx DSP Scan Workspace.

1. **Open the ROIs List** (see Figure 43).

2. Select the **Export All** button from the ROIs List to export all ROIs for custom segmenting. Alternatively, to export one individual ROI, use the **Export** button below the ROI of interest.

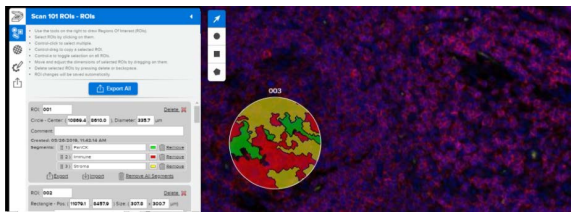


Figure 43: Scan Workspace ROIs List with Export Image link

3. The system will export files in multi-channel TIFF format. Save to a desired location.
4. **Use the instructions and tools in the external program** to create bitmasks within the exported ROI. See the **GeoMx DSP ImageJ Guidelines** at https://www.nanostring.com/geomx-online-user-manual. Segments cannot overlap.
5. **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 0 in your saved image will be treated as segment area. 0-value pixels will be treated as empty tissue space. Return to the Scan Workspace
6. Select the **Import** button below each ROI of interest to individually import the applicable custom segment mask.
7. **Browse to location of your saved image and select Open.** The segments will appear on the selected ROI.

9 Make changes (if needed)

You may occasionally need to revisit the Scan Workspace to make changes to your ROIs before collection. Or, you may need to rescan your slide due to focus issues.

EDITING ROIS

Once you have placed the ROI, you may adjust it. Select the **select/edit ROI** button from the Scan Workspace **(see Figure 44)** and click on the shape you'd like to edit.

- Click and drag the center point of the shape to move the shape.
- Click and drag a white perimeter point to resize the shape.
- To segment ROIs, see **Segment ROIs (if applicable) on page 49**.
- 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.

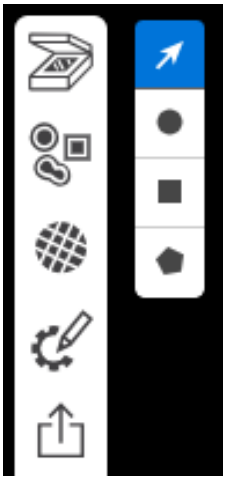


Figure 44: Scan Workspace select ROI button

EXPORT IMAGES

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

1. Select the **Export** menu in the Scan Workspace.
2. The default view is **Rendered Scan Image** (see Figure 45); choose this for a full-color publication-quality image.
   - Choose **RawChannel Images** (see Figure 46) to export single-channel, high-resolution images. This exports the raw, undoctored tiff file and may be several GB in size.
   - 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.

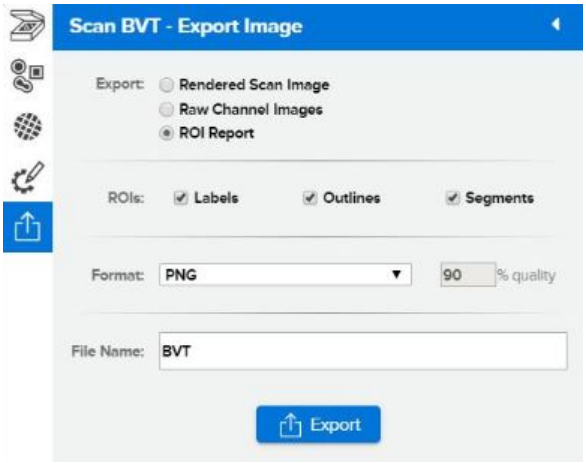


Figure 45: Export image menu - rendered scan

The *.tiff* image format currently does not display properly in the HTML report when viewed in the Google Chrome browser.

3. Use the **View** and **ROIs** fields to distinguish what to export on the scan.
   - **Rendered Settings** 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 (*.jpeg*, *.png*, *.tiff*).
   - The *.jpeg* format allows you to adjust the file compression in the **% quality field**.
   - The full exported image will not be zoomed and will reflect the **Render Settings.**

5. Enter the **File Name** you'd like to use for the image.
6. Select **Export.**

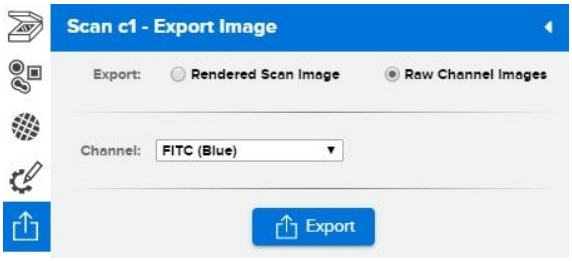


Figure 46: Export image menu - raw tiff

10 Exit Scan Workspace and Approve ROIs

1. **Select the Exit button in the lower left** of the Scan Workspace toolbar (see Figure 47) when ROI selection is complete.

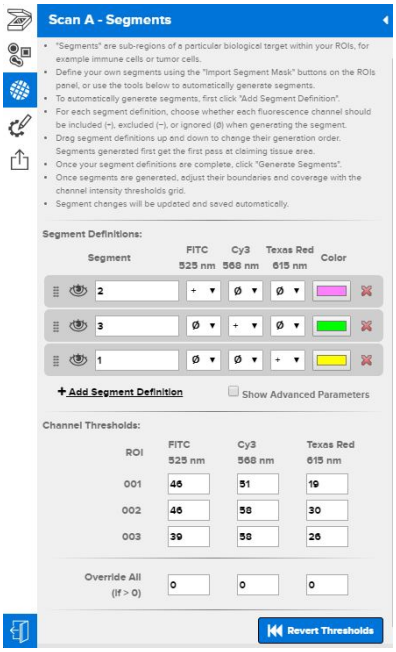


Figure 47: GeoMx DSP Scan Workspace with Exit button at lower left

> **IMPORTANT:** If you made changes to segment definitions, ensure that you have clicked the **Save Changes** button before exiting the Scan Workspace. If you do not do this, the changes to the segment definitions will not be applied to the scan.

2. A pop-up window will ask you to **Approve ROIs** or **Save and Exit**. Approve the ROIs to queue them for collection. Save and Exit to save the ROIs but abstain from Collection at the current time. The Scan Workspace will close and you'll be returned to the slide view of your DSP run.

> **IMPORTANT:** Do NOT attempt to collect ROIs from a scan with poor focus.

3. **Repeat steps 7 - 10 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**(see Figure 48). You may modify ROIs until it is time for them to be collected.

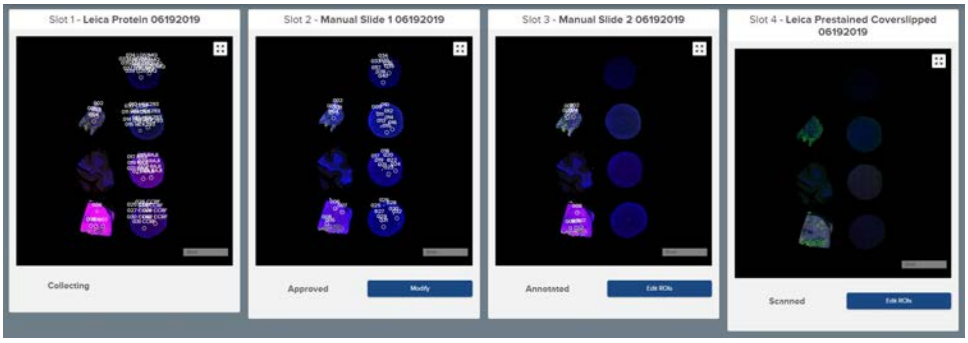


Figure 48: Scan status messages and buttons

Once the GeoMx DSP System has begun to collect from a slide **(see Figure 49)**, its ROIs can no longer be modified and this button will not be available.

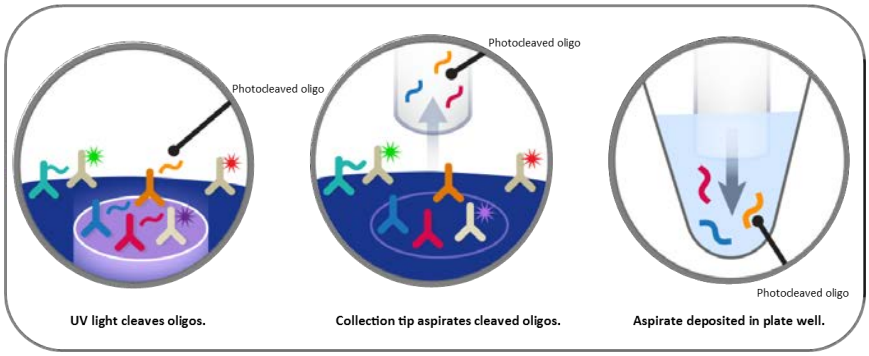


Figure 49: ROI collection on GeoMx DSP

- If the collection plate becomes full, the GeoMx DSP instrument will pause for you to replace the full collection plate with a new one. Follow the instructions in the GeoMx DSP software to change the collection plate.

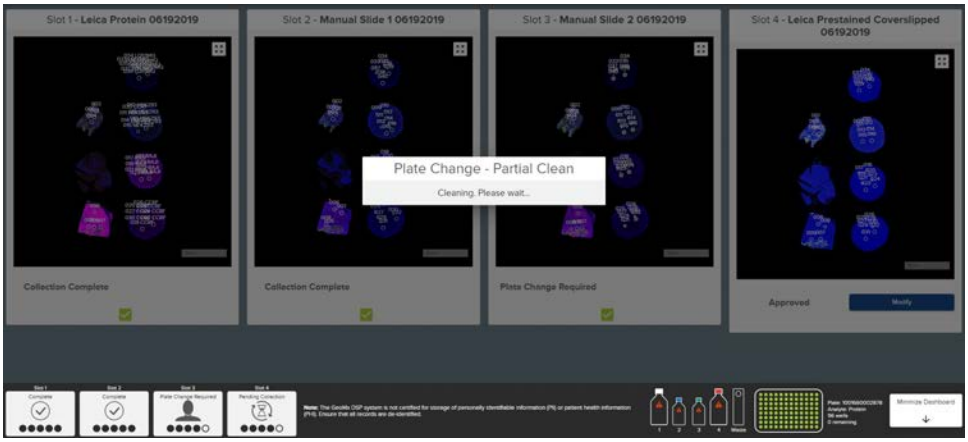


Figure 50: Plate Change notifications

- When all ROIs for each slide have been collected, the system will move on to the next stage. See **Complete on page 63**.

Complete

Once ROI collection is complete, the system will initiate an **Instrument Cleaning** process. Each scan image will have the status **Collection Complete** displayed.

Choose the **New Data Collection** button to initiate a new run using either the same slides or same plate. Choose the **Remove Slides and Microplate** button if not reusing either component.

11 Finalize and unload the GeoMx instrument

REMOVING THE COLLECTION PLATE

Follow the prompts in the GeoMx DSP Control Center to remove the collection plate from the instrument **(see Figure 51)**.

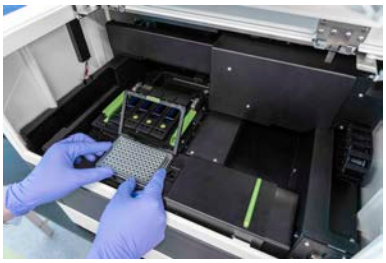


Figure 51: Unloading the collection plate

After the collection plate has been removed from the DSP instrument, it should be immediately prepared for the nCounter run (see the **GeoMx DSP nCounter Readout User Manual**) . Alternatively, if using the following safe storage guidelines, plates may be stored for later use. Deviating from the safe storage guidelines may result in reductions in data quality.

If not immediately preparing your DSP collection plate for readout, the plate must be sealed with adhesive foil to prevent contamination, and adhere to the following guidelines:

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

Removing the Slide Holder

1. Follow the prompts in the GeoMx DSP Control Center to remove the slide holder from the stage (see Figure 52).
2. Using a pipette, remove and dispose of the buffer from each slide in the slide holder.
3. In a designated laboratory space, open each slide slot clamp and unload each slide.

Figure 52: Unloading the slide holder

4. Store the slides:
   - Store **protein slides in TBST** at 4°C (in a dark container) for short term storage (1 - 3 days) or in mounting media for long-term storage (>3 days; see next section).
   - Store **RNAslidesin2xSSC** for up to 48 hours.
5. Clean the slide holder. See Cleaning the Slide Holder. Store the slide holder outside of the GeoMx instrument.
6. Follow the prompts to close the instrument door.

Slide Mounting Procedure for Long-Term Storage (>3 days) for Protein Slides

1. **Rinse slide** to be mounted with TBS-T or PBS-T, touch the edges of slide on 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 if necessary to ensure the slide does not dry out and there is adequate tissue coverage. Optional: let stand approximately 5 minutes.
3. **Mount coverslip** (hold one slide to align, then drop from one side to the other) and remove excess mounting medium.
4. **Allow slide to dry** at ambient temperature in a dark area overnight (bench drawer).
5. Store slide at ambient temperature or at 4°C. Avoid light exposure.

Your samples are now ready for processing on an nCounter platform. See the **GeoMx DSP nCounter Readout** user manual.

FINALIZING THE COLLECTION PLATE

Finalization of the collection plate confirms the totality of the samples that will be hybridized and counted together; wait to finalize your plate if you intend to add more samples to the present nCounter run set. If you finalize this run and later collect more samples in this same collection plate, you will repeat this process, generating a new CDF and worksheet, and hybridize and count this set separately from the previous.

Notes about finalizing your plate:

- Plates are **finalized by the row**, not by the well, even if not all of the wells in a row have been used.
- Once you've finalized a set of rows, you **cannot add more unfinalized rows to that finalization**. For this reason, if you intend to add additional aspirates to a collection plate to pool with the existing ones for a common nCounter run, do not finalize the collection plate until they have all been collected.
- You can re-insert the plate and use the unused rows. You can also always access the lab worksheet and CDF.

1. Double-click on the plate icon area at the lower right of the GeoMx DSP Control Center (see Figure 53). If the plate is not currently on the instrument, enter its barcode.

Figure 53: plate icon in GeoMx DSP Control Center

2. The **Finalize Plate** window will appear.

   The **Finalize Plate** window (see Figure 54) will provide you with a **Readout Group Information** table, summarizing the status of each row of the collection plate.

Figure 54: Finalize Plate window

- Enter the **GeoMx Hyb Code Pack** lot number to be used in the downstream nCounter processing and click the **Update** button. This information is needed for the system to identify the appropriate calibration data, but can alternatively be entered when counts are imported. Find the lot number on the Hyb Code box (see Figure 55).If you do not know the lot number at this stage, you may skip this field and enter it when you upload nCounter counts.

Figure 55: Hyb Code Lot Number

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

IMPORTANT: If receiving counts/readout from an off-site location, ensure they return with the Hyb Code lot number used in conjunction with the readout run.

- If you have just completed your 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**.

3. Select the **Finalize** button.
4. Insert a portable USB drive into the front USB port of the GeoMx DSP instrument to store the files.
5. Select the **Download** button in the **Definition File** category; this will initiate the download of your CDF (for the MAX/FLEX platform). Your files will be saved on your USB drive. You will transfer this to your nCounter MAX/FLEX platform (see the **GeoMx DSP nCounter Readout User Manual**).

IMPORTANT: Do not edit the contents of the CDF file.

6. Select the **Download** button in the **Library Prep Information** category; this will initiate the download of a worksheet to use for your reference during hybridization setup. This worksheet also contains information needed to set up an nCounter SPRINT platform.

Creating Slide Records

Slide records can be created at any time before or during the DSP workflow. To create a slide record during the DSP run workflow, see **Identify Slides on page 32**.

CREATING A NEW FOLDER

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

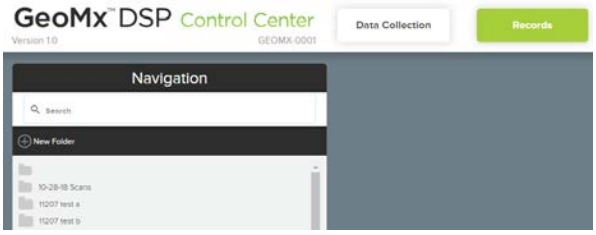


Figure 56: GeoMx DSP Control Center Navigation window under Records

1. Select the **Records** button in the DSP Control Center **(see Figure 56)**.
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.

CREATING A NEW SLIDE RECORD AS A PRE-WORK STEP

To create a slide record outside of the GeoMx DSP workflow:

1. Select the **Records** button in the DSP Control Center (see Figure 57).
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.

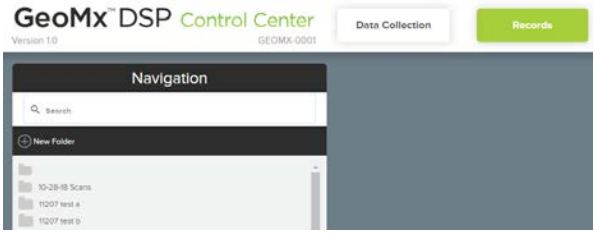


Figure 57: GeoMx DSP Control Center Navigation window under Records

4. The **Creating new slide record** window will appear to the right of the Navigation window. Fill in:
   - **Slide Label**: the unique identifier which will show in the Navigation window
   - **Barcode** (optional)
   - **Description** (optional)
5. Select **Save New Slide**.

SLIDE RECORDS THAT DO NOT YET HAVE ASSOCIATED SCAN PARAMETERS

If the selected slide record does not yet have a set of scan parameters associated with it, the Scan Card will simply read, "No scans associated with this slide" (see Figure 58). To remedy this, click the **Add Scan** button in the **Slide Record** window (see Creating Scan Parameters on page 69).

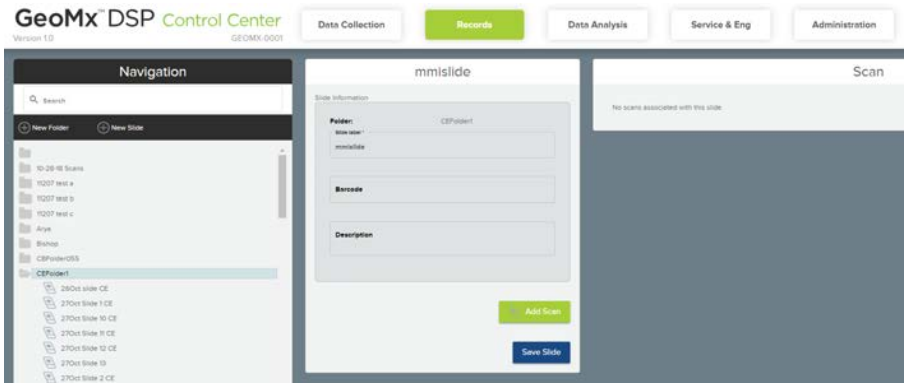


Figure 58: Slide record under Records in GeoMx DSP Control Center

Creating Scan Parameters

You may choose your scan parameters when creating a new slide record or associating a loaded slide with an existing slide record. See **Create: on page 34** to create a slide record during the GeoMx DSP run or **Creating Slide Records on page 67** to create a slide record before the run.

1. **Enter a Name** for this scan. This should be a unique identifier.

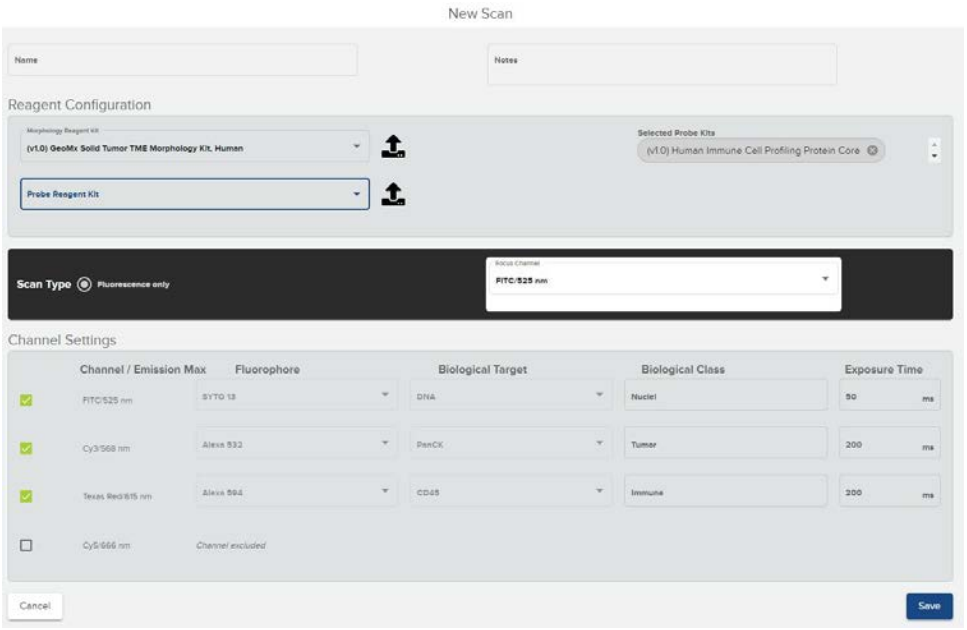


Figure 59: Scan Parameters

2. **Upload/Select Reagent Configuration files.**

   GeoMx DSP configuration files associate RNA and Protein assay targets with GeoMx readout barcodes. Morphology Kit configuration files are used to auto populate key target information for ROI selection in the GeoMx DSP Software. Find the files for the kits used on your slide at www.nanostring.com/dspconfigfiles. Save these files to a USB drive.

   - Select the **Morphology Reagent Kit** used in preparing the slide from the drop-down. If not already loaded, select the **upload** button to the right of this field to upload the respective configuration file from your USB drive (see above). See **Example Scan Parameters on page 72** for GeoMx Solid Tumor TME and Melanoma Morphology parameters.
   - Select any probe kits used in preparing the slide from the **Probe Reagent Kit** drop-down. Select the **upload** button to upload configuration files from your USB drive, if needed. Any selected probe kits will be listed to the right of the upload buttons. The Core Kit must be selected before the compatible Module Kits will be listed.

   IMPORTANT: ***Select the file that matches the name, lot number, and Probe R space of the GeoMx Assays used.*** Ensure you have uploaded the correct configuration files for the core and each module used during slide prep. 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).**
   - Proceeding through aspirate collection with **an incorrect *.pkc* file** may result in a **permanent misassociation of probes** and invalid data displayed in Data Analysis.
   - For any Substitute Probe R (**Sub Probe R**) used in **Protein nCounter** slide preparation, a **Sub Probe R configuration file** must be used. See Appendix V of the **GeoMx-nCounter Slide Preparation User Manual** for more information on using a Sub Probe R.
   - If spiking in **custom targets** to an existing GeoMx assay, NanoString's bioinformatics department will provide a *.pkc* file to be uploaded in the Probe Reagent Kit field in addition to the GeoMx assay *.pkc*.
   - If adding **custom RNA targets to an nCounter** assay, only one custom RNA module should be used per scan.
   - If a protein module added to the list shares the same reporter space as another module on the list, a warning will appear; carrying forward with this scenario poses the risk of losing data from both modules. Remove one of the conflicting modules.
   - If you do not see the file you need, contact geomxsupport@nanostring.com for assistance.

Configuration files contain probe group designations which will materialize in the Data Analysis stage.

3. Select the appropriate **Scan Type** and **Focus Channel** for your slide.
   - The scan type option will be set to **Fluorescence** only; the **Brightfield only** and **Fluorescence and Brightfield** options are inactive at this time.
   - Focus channel options (for fluorescence): **FITC / 525 nm**, **Cy3 / 568 nm**, **Texas Red / 615 nm**, and **Cy5 / 666 nm**. The **nuclear dye** (default in the **FITC/525 nm** channel) is typically used as a focus channel.
4. Verify the **Channel Settings** fields.
   - Ensure all channels you wish to use in the scan have been checked.
   - Adjust the value in the **Exposure Time** field, if desired.
   - Adjust the name in the **Biological Class** field, if desired.
5. Fill in the custom morphology field (if applicable) to populate custom fluorescence exposure settings.
6. Select **Save**.

EXAMPLE SCAN PARAMETERS

SCAN PARAMETERS: GEOMX MELANOMA TME MORPHOLOGY KIT, HUMAN

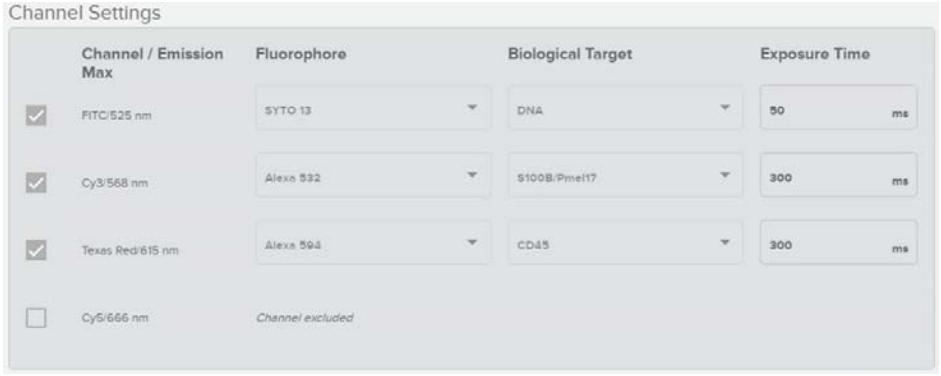


Figure 60: Example of GeoMx Melanoma TME Morphology scan parameters

SCAN PARAMETERS: GEOMX SOLID TUMOR TME MORPHOLOGY KIT, HUMAN

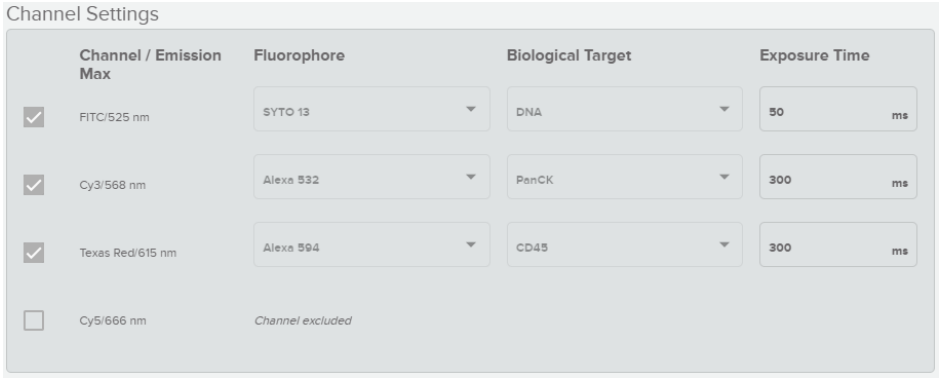


Figure 61: Example of GeoMx Solid Tumor TME Morphology scan parameters

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 window **(see Figure 62)** shows the hierarchy of any established slide records, scan cards, and Data Analysis studies. Use the search bar to filter records, if desired. In this window you may:

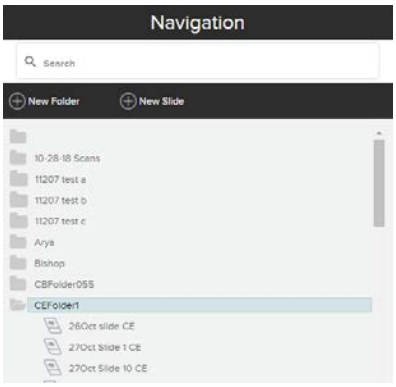


Figure 62: Navigation window under Records

- 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.
- 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.

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.

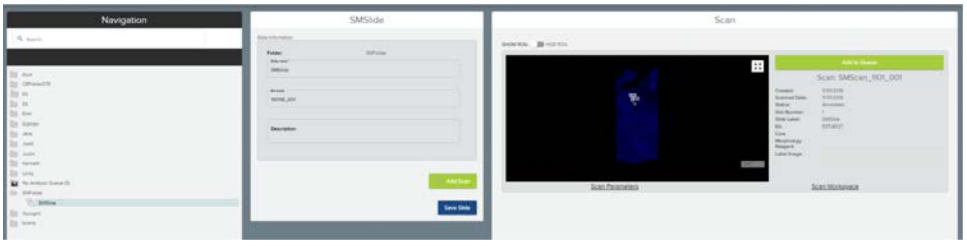


Figure 63: Navigation window, slide record, and scan card

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

- **Hide or show ROIs** using the slider.
- **Scan Parameters** to view the Scan Parameters window.
- **Scan Workspace** to open the Scan Workspace window. Here, you may perform tasks such as establishing ROIs or exporting images (see **ROI Selection & Collection on page 41**).

> ROIs may only be selected if the slide is currently loaded on the instrument and has yet to be collected.

The listed **Status** for the scan will inform on what stage it is in according to the GeoMx system. Possible statuses are: **Annotated**, **Aborted**, **Collection Complete**, and **Readout Complete**.

If the scan's status is **Readout Complete** (the scan's counts have been returned from the nCounter system), the **Add to Queue** button will be functional in the upper right corner of the scan card. Click the **Add to Queue** button to send this scan's counts to the Data Analysis module. The number of scans in the Data Analysis Queue will be represented by a number on the **Data Analysis** button on the GeoMx DSP Control Center header. See the **GeoMx DSP Data Analysis User Manual**.

MANAGING SCAN CARDS

Select a folder from the Navigation window. Click the grid button **(see Figure 64)** or the bars button to see alternative **gallery views** of the scan cards in that folder **(see Figure 65)**.

Figure 64: Gallery view and list view buttons

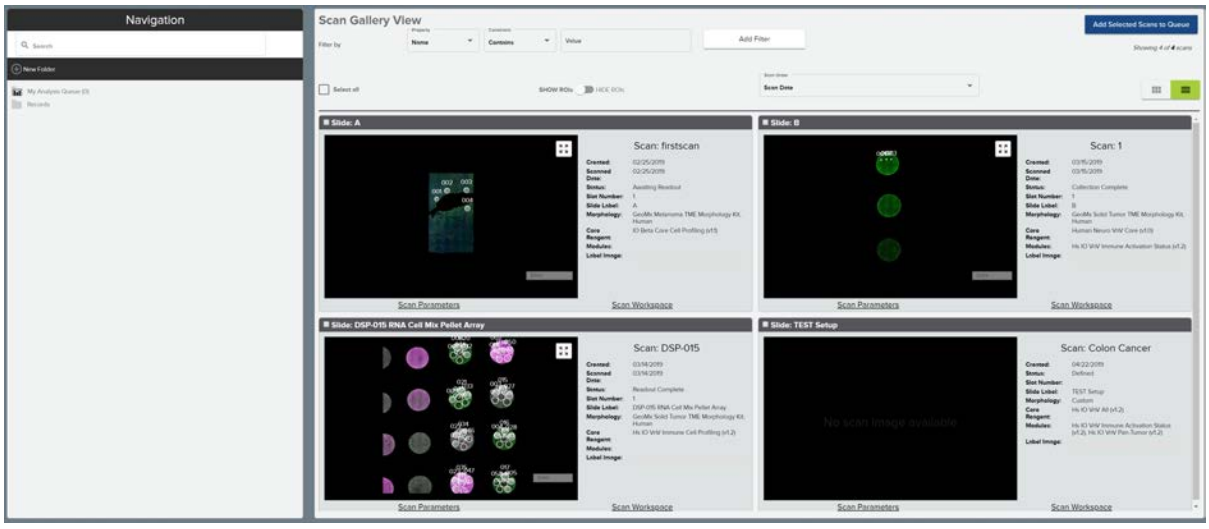


Figure 65: Scan gallery view

FILTER BY

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

- **Property**: Name, etc.
- **Constraint**: contains, equals, between, etc.
- **Value**: customizable field.

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 drop-down (Scan Name or Slide Name), if desired.

Once all desired scans have been selected, you may select the **Add selected scans to queue** button in the upper right corner to add selected scans to the data analysis queue. The number of scans in the Data Analysis Queue will be represented by a number on the **Data Analysis** button on the GeoMx DSP Control Center header. See the **GeoMx DSP Data Analysis User Manual**.

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 **Creating a New Folder on page 67**.
- Select **Edit** to edit the highlighted folder.
- Select **New Slide** to create a new slide record under the highlighted folder. See **Creating a New Slide record as a pre-work step on page 68**.
- Select **Delete** to delete the highlighted folder. Only empty folders may be deleted.

Administration

The **Administration** button in the DSP Control Center will be active for both Administrative and General users. Administrative users will be able to: access user and group accounts, shutdown the instrument, update the software, download logs, reset errors and warnings, and access other system details **(see Figure 66)**. General users will only have access to the Download Logs feature of the Administration module.

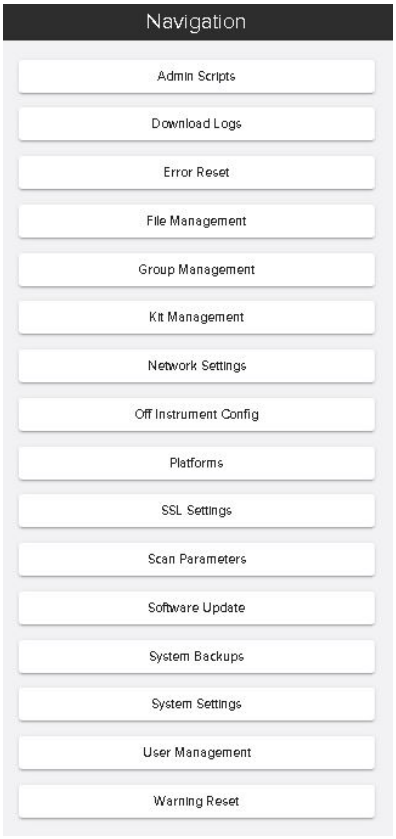


Figure 66 : GeoMx DSP Administrative menu

ADMIN SCRIPTS

Admin Scripts allows you to run individual scripts for specific purposes. Contact NanoString (geomxsupport@nanostring.com) before running any of these scripts.

- **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). Only run this at the instruction of NanoString personnel.
- **ADMIN_RestoreSystemAfterWastePumpFlush.xaml** 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. Only run this at the instruction of NanoString personnel.
- **ADMIN_SystemPrimeFromBottle.xaml** is for recovering from a fluidic error on the needle if it has pulled air into the system. Only run this at the instruction of NanoString personnel.
- **ControlDoor.xaml** lets you unlock/lock door. A prompt will appear to ask if you want to lock the door. Type **True** or **False**.
- **RestartInstrumentService.xaml** restarts the instrument services.

DOWNLOAD LOGS

Select the day range of interest (see Figure 67). You may select one or more days in a continuous range for which to extract logs.

Figure 67: Download Logs tab under Administration

- If logged into the instrument directly, insert a USB drive. All relevant log files (system audit log, file server logs, etc.) will be exported as a set and should have filenames which 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.
- Logs are exported to the remote user's normally defined download destination.

FTP uploads and downloads are logged by the FTP server process in a separate file.

ERROR RESET

An administrative user can access this option through the Administration button in the GeoMx Control Center.

> **IMPORTANT:** It is recommended that you contact geomxsupport@nanostring.com before attempting this option.

Clicking on the **Reset Error Level** button results in a 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.

File Management

Select File Management to set archive settings (see Figure 68).

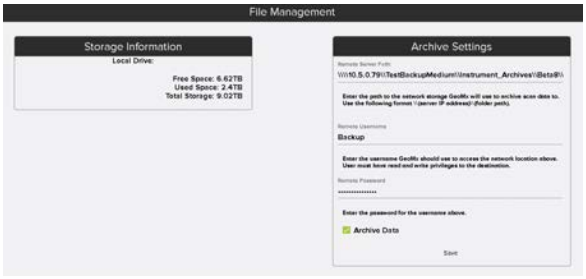


Figure 68: File Management tab under Administration

- **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 start with four backslashes, then the IP address and folder path in this format, using backslashes (\ ), not forward slashes (/):

  \\\\server IP address\\folder path

> Uploads from the GeoMx DSP System may occur at any time; the remote location must be available at all times.

- **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. Exclude $ and ! and / in passwords.

Toggle the Archive **On/Off** button at the bottom of the form to **On**.

Click the **Save** button.

A confirmation or error message will appear. This message may take several seconds to appear.

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 geomxsupport@nanostring.com to troubleshoot.

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

GROUP MANAGEMENT

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

Enter a Group Name and click **Create New Group** (see Figure 69).

For an existing group, select **Rename**, **Manage Members**, or **Delete**.

Figure 69: Group Management tab under Administration

RENAMING A GROUP

1. Once on the **Group Management** tab under **Administration**, click the **Rename** button next to the group of interest.
2. Enter the new name and select **Save**.

MANAGING MEMBERS

1. Once on the **Group Management** tab under **Administration**, 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.
   - To add an available user to the group, highlight them in the **Available Users** field and select the **Add Users** button.
   - To remove a user from the group, highlight the individual in the **Users in Group** field and select the **Remove Users** button. A confirmation message will appear; select **Save** to complete the removal.

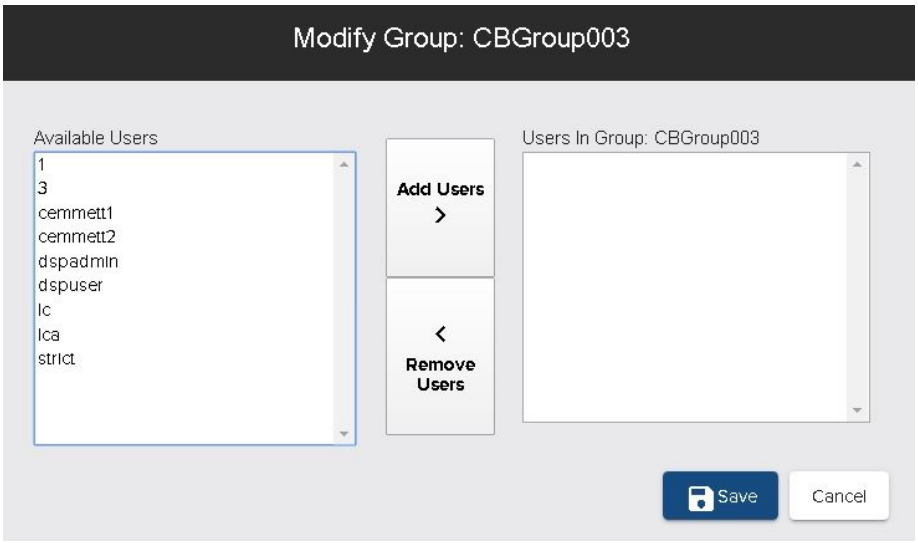


Figure 70: User group management window

DELETING A GROUP

1. Once on the **Group Management** tab under **Administration**, click the **Delete** button next to the group of interest.
2. **Confirm** to complete the deletion.

KIT MANAGEMENT

Use this page to upload configuration files to your system. Download configuration files from www.nanostring.com/dspconfigfiles.

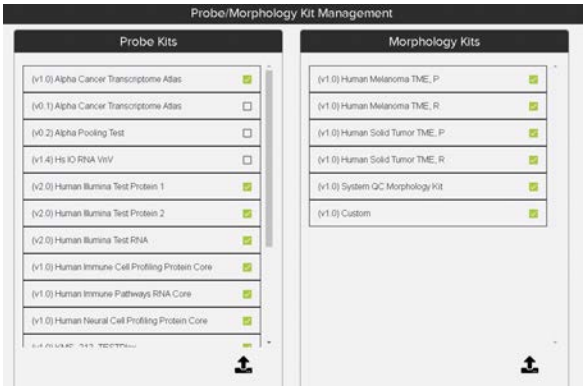


Figure 71: Kit file management window

NETWORK SETTINGS

The Network tab allows you to adjust network settings (see Figure 72). Select **Dynamic** or **Static IP Address** from the drop-down menu.

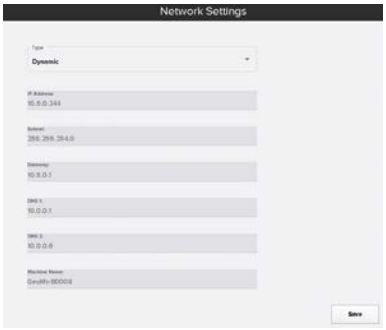


Figure 72: Network tab under Administration

Instructions to remotely connect to your DSP instrument

This will allow you to do data analysis, ROI selection, and export images remotely.

1. Connect the GeoMx DSP instrument to your network with a network cable.
2. Log in 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.

   If you do not see an IP-address here now, you may need to reboot the system (shut down from the menu, then switch off with the switch in the back, then after 10-20 seconds, switch back on).

6. From your remote computer, open a Chrome browser (other browsers may not be optimal).
7. Browse to https://geomx-#### where #### is the GeoMx **Machine Name** you noted from the Network Settings window. In the example from this figure (see Figure 72), the address would be *https://geomx-dev7*.
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 from this figure (see Figure 72), the address would be *https://10.0.0.6*.
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.

OFF INSTRUMENT CONFIG

You have the option to install the GeoMx DSP Data Analysis Suite on a server separate from the GeoMx DSP instrument. Contact your NanoString representative for details. Once this software has been installed on an external server, you must return to the GeoMx system to configure it. Once in the GeoMx DSP Control Center, access the **Off Instrument Config** tab under **Administration**.

Ensure that there are no Data Analysis sessions in progress when configuring off instrument Data Analysis; any sessions in progress will be terminated.

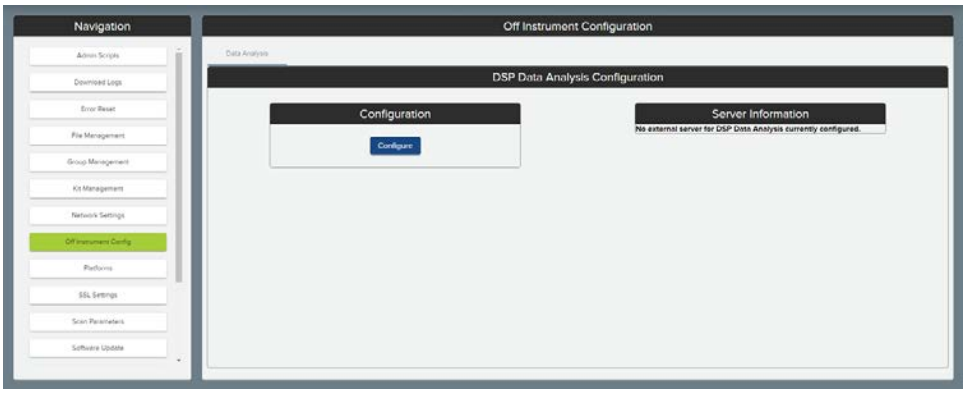


Figure 73: Off Instrument Configuration window

1. Click the **Configure** button (see Figure 73).
2. Input the **IP Address** of the server. This should be a Static IPV4 address. Click **Next**.
3. 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.
4. Enter the **six digit pin** provided to you during the installation of the **Off Instrument Data Analysis** software. Contact your NanoString representative if you do not know this number. Click **Next**.
5. You will be asked to **confirm the configuration**. This is the last opportunity to abort the configuration. Click **Continue**. Setup does not take very long. Data Analysis will be temporarily taken offline while setup continues.
6. When the **Setup Complete** window appears, click **OK**.
7. The server availability and address will be displayed (see Figure 74). If this information does not appear, refresh the screen.

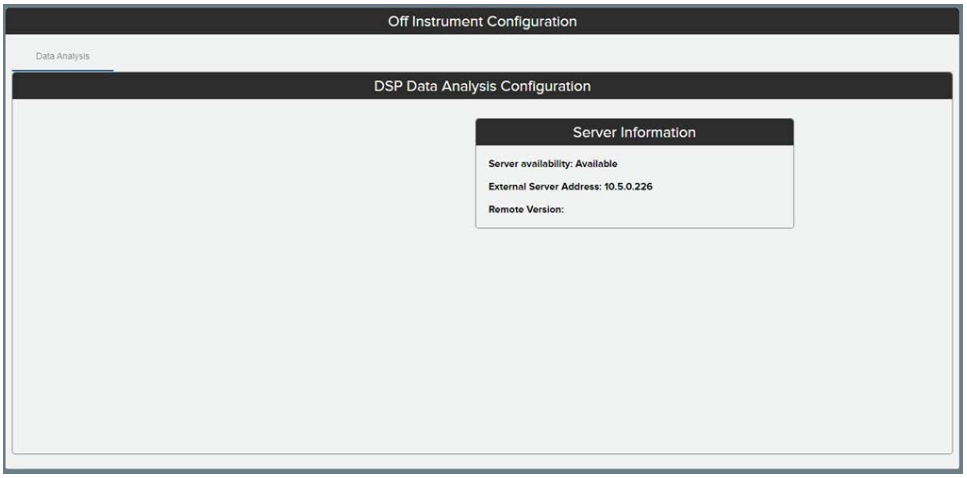


Figure 74: Off Instrument Data Analysis server information window

PLATFORMS

Use this window (see Figure 75) to select the nCounter platform, Illumina NGS platform, or both, according to what platform(s) your laboratory will use.

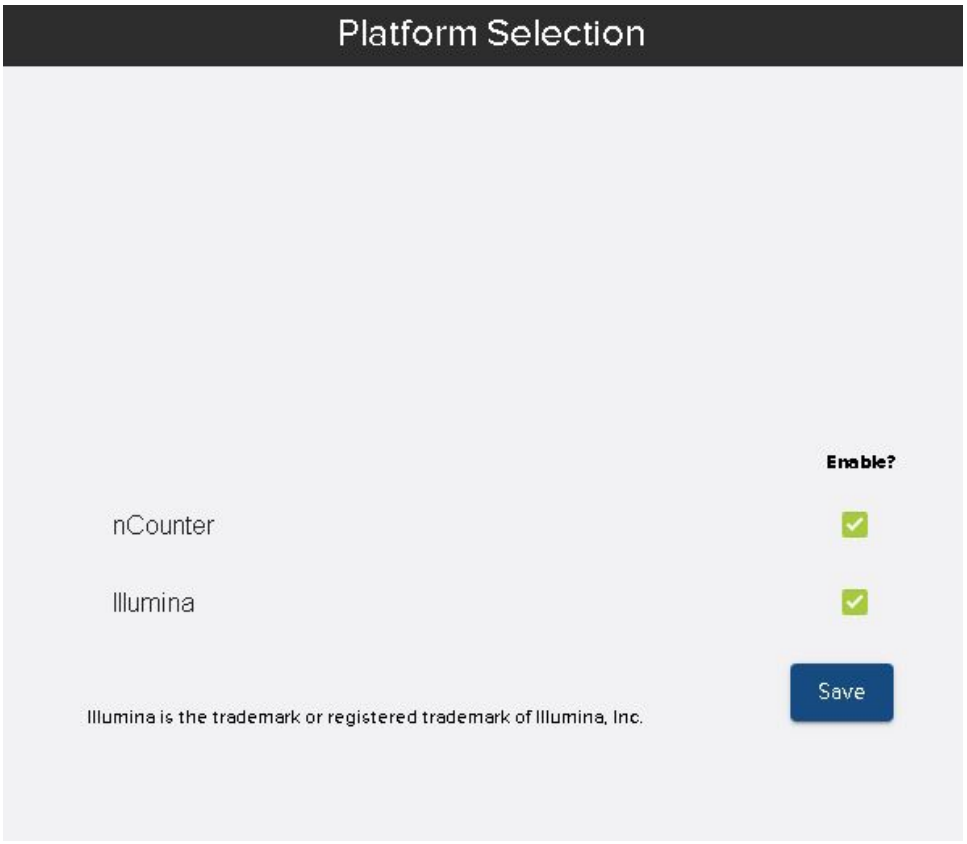


Figure 75: Platforms window

SSL SETTINGS

If your browser does not recognize the GeoMx DSP software security certificate and warns that the site is "not secure," you may follow your browser's prompts to proceed anyway. Once in the GeoMx DSP Control Center, access the **SSL Settings** tab under **Administration**. Click **Download** to download the security certificate. Follow the instructions below to install the certificate. Certificates expire periodically; you may need to repeat this procedure to download and install an updated certificate in the future.

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

NanoString insists upon secure HTTPS connections for three main reasons:

- **Confidentiality**. This protects the communication between your instrument and remote connections from workstations for sending passwords, browsing images or selecting ROIs. Without using HTTPS, someone on the same network could potentially see private

information being sent over the network.

- **Integrity**. This makes sure information reaches its destined party in full and unaltered.
- **Authentication**. This ensures that the instrument you are connecting to is what it claims to be. This helps potential intruders from pretending to be an instrument so that you will send them sensitive information such as your username and password.

This is only relevant for ancillary computers being used to remote into the DSP. This process is not necessary for the on-board DSP computer.

1. **Login** to the GeoMx system as an Administrative user. Click on the **Administration** button and select the **SSLSettings** button from the menu.
2. If your system is not networked, insert a USB drive into the GeoMx instrument. If your system is networked, you can use the network to transfer the certificate file to your remote computer.
3. Click **Download** to download the security certificate (see Figure 76). 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**.

Figure 76: SSL certificate download

4. Use the USB drive or your network to **transferthecertificatefiletoyourremotecomputer** and save it in an accessible location.
5. **Navigatetothecertificate**, double click on it, and select **Open** (see Figure 77).

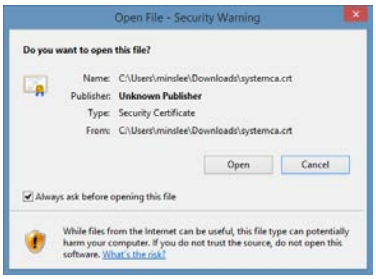


Figure 77: Open file window

6. **Click** the **InstallCertificate** button (see Figure 78).

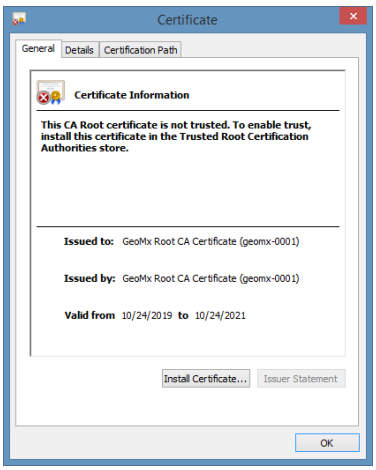


Figure 78: Install certificate window

7. **ChooseLocalMachine** and click **Next**(see Figure 79). Administrative privileges are required for this step; you may need to contact your IT department.

Figure 79: Certificate import wizard - local machine

8. Select the radio option for **Place all certificates in the following store** , then click **Browse**(see Figure 80).

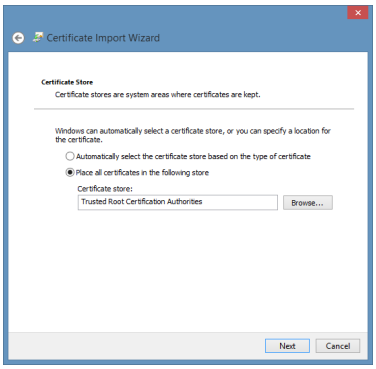


Figure 80: Certificate import wizard - trusted root certification

9. **Browse** to **Trusted Root Certification Authorities** and click **OK** (see Figure 81).

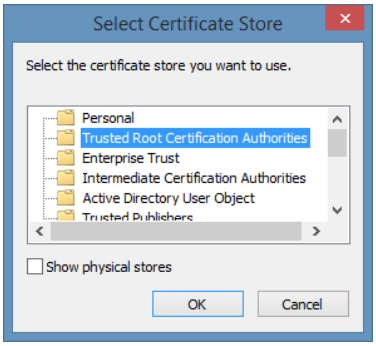


Figure 81: select certificate store

10. Complete the remainder of the **Certificate Import Wizard** steps until you receive a successful message.
11. 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**).

SCAN PARAMETERS

Manage and create new fluorophores and biological targets to be available for custom analysis in the **Scan Parameters** window.

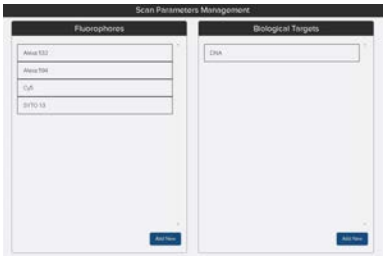


Figure 82: Scan Parameters management window

SOFTWARE UPDATE

> **IMPORTANT:** Do not attempt to update software while a run is in progress.

GeoMx DSP software updates consist of multiple *.cab* files. Save these files in a folder and place them on a USB drive. Ensure the instrument is in an Idle State before attempting to update the software.

IDLE STATE PAGES

There are two pages that are considered idle. One is the **Collection Complete** page which includes two blue buttons. The second page is the **Replace Plate** page. If you are not at either of these pages, you will not be able to update the software. Below are instructions to move the system to the **Replace Plate** page.

Figure 83: Collection Complete idle state

> **IMPORTANT:** If you are on an idle screen, and system still prompts that it is not idle, power cycle the instrument, click **New/ContinueRun** and try again.

IF A PLATE IS ALREADY INSIDE FROM THE PREVIOUS RUN:

1. Use the **Assist wrench** button and restart the workflow if stalled in **PreparingInstrument**.
2. The **Replace Plate** window will appear. The instrument is now in an idle state – do not click anything in this window.

Figure 84: Replace Plate idle state

3. Continue with the software update from the **ReplacePlate** screen.

IF NO PLATE IS INSIDE YET:

1. Start a new run, loading the instrument with a plate holder and plate.
2. After closing the door, continue to **PreparingInstrument**.
3. Use the **Assistwrench** button and restart the workflow.
4. The instrument will go back to a new run and the **ReplacePlate** window will appear. The instrument is now in an idle state – do not click anything in this window.
5. Continue with the software update from the **ReplacePlate** screen.

UPDATING GEOMX DSP SOFTWARE FROM V1.4 TO SUBSEQUENT VERSIONS

1. **Log in** to the GeoMx DSP system as an Administrative User. Ensure the instrument is in an ***Idle State*** before attempting to update the software. If stalled at **Preparing Instrument**, click on the **Wrench Assist** icon and click **Restart**.
2. From another computer (not the GeoMx instrument), **download** the zipped software update file. Unzip the file by right-clicking and selecting **extract all**.
3. **Save the unzipped .cab files** to the root of an otherwise-empty USB drive (at least 16 GB). **Safely eject** the USB from the computer.
4. **Insert the USB drive** into the GeoMx DSP instrument
5. **Log in** to the instrument as an Administrative user (if you haven't already), click the **Administration** button from the GeoMx DSP Control Center, then select the **Software Update** option.
6. Click the **Software Update** button. The instrument will update using only the .cab files in the root directory. This may take approximately 5-10 minutes; do not remove the USB drive during this time.

   The screen may switch to several screens, toggling between the Google Chrome page, a black page and the DSP Logo screen with a green loading bar at the top; wait for it to go back to the original state.

7. When the GeoMx DSP log in page appears again, log in with Administrator privileges.

   If the screen goes solid grey and the log-in page does not display automatically, refresh the page. To do this, use keyboard shortcuts Ctrl+R or F5.

8. Check the GeoMx DSP software version number in the upper left of the Control Center; it should now display the new version number. You may now remove the USB drive from the GeoMx instrument.

System Backups

Once an administrative user has provided a network path for archiving (see **File Management on page 80**), the system should automatically start archiving data and performing backups. The dates and location of all backups will be listed in here.

System Settings

Use this menu option to shutdown the instrument. See **DSP system shut down**.

USER MANAGEMENT

The **User Management** window allows you to edit or create new user profiles **(see Figure 85)**. Scroll to the bottom of the **User Accounts** list and check the **Enforce Strict Passwords** box to require more complex passwords. With this box checked, each user password created from this point forward will need to be at least 14 characters, containing at least three of the following: uppercase letter, lowercase letter, number, and special character.

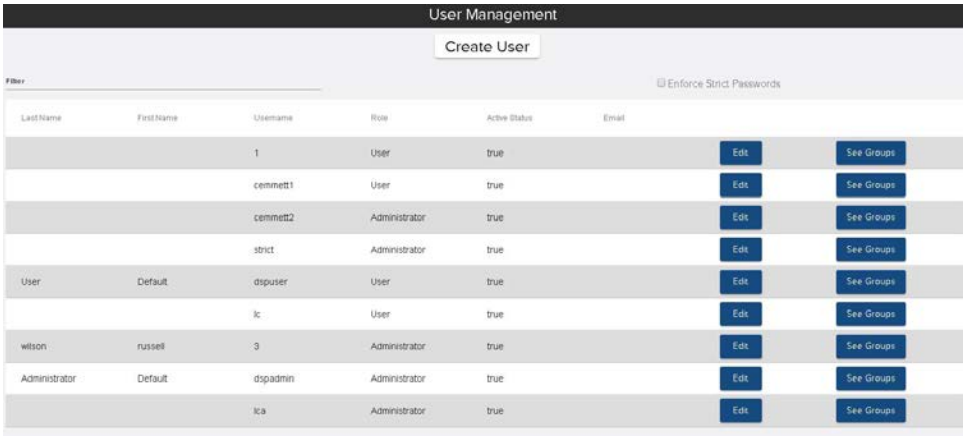


Figure 85: Manage Users tab under Administration

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.**

ADD USER

1. On the **User Management** tab under **Administration** , select the **Create User** button.
2. The **Create New User** window will appear (see Figure 86); fill out the fields.
   - Create a username and password for the new user.
   - 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).

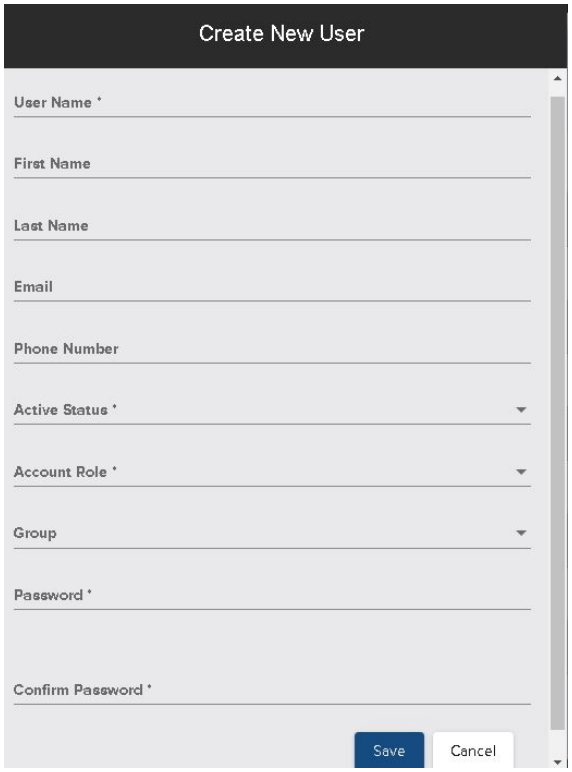


Figure 86: Add Users tab under Administration

> 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**.

WARNING RESET

Figure 87:

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

System Startup & Shutdown

The GeoMx DSP instrument should be shut down and restarted on a weekly basis. Only a user with administrative privileges can initiate the shutdown process. The power button is located on the back of the instrument (see Figure 88).

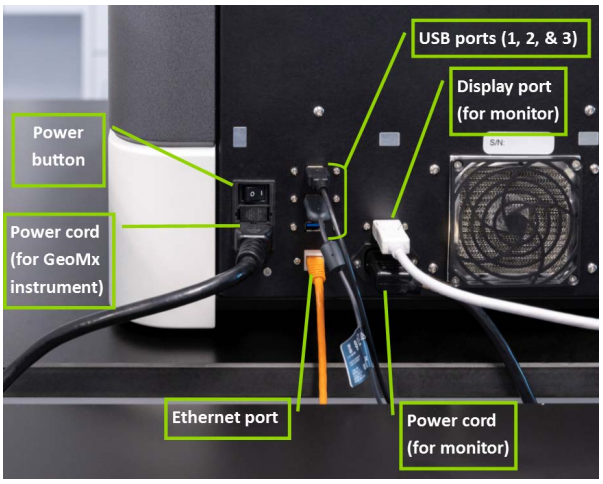


Figure 88: GeoMx DSP backside diagram

DSP SYSTEM 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; you may log in locally or remotely. See **Creating & Managing Users.**
3. Once you have logged in, proceed to **Data Collection** to start a run or click **Records** to manage or create new slide records.

DSP SYSTEM SHUT DOWN

The GeoMx DSP instrument should be shut down and restarted on a weekly basis.

1. A user must be logged in locally as an administrator in order to shut down the instrument.
2. Select the **Administration** button from the GeoMx DSP Control Center.
3. Select **System settings**, then **Shut Down DSP**.
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 appear, instructing 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," you may turn the power button **off**. Wait at least 60 seconds to reintroduce power if power cycling.

   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.

GeoMx DSP Instrument Reagents

Figure 89: GeoMx DSP instrument reagent bay diagram

The GeoMx DSP instrument requires the following reagent bottles in the reagent bay (see Figure 89):

- **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:** Ensure the instrument is in an idle state before changing reagents (see Idle State Pages). In addition, DO NOT top off any reagents. Rather, replace entire bottles through the wizard when needed.

CHANGING REAGENT BOTTLES

Checking the reagent and waste levels is an important step prior to starting your GeoMx DSP run. The system will check 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. The instrument must be idle in order to remove a reagent bottle; during a run, the instrument is only idle during the **Replace Plate** phase and the **Collection Complete** phase. Click on the **Reagent Status Indicator** in the GeoMx DSP Control Center to initiate the bottle change process. Follow the prompts in the GeoMx System.

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 bottle cap by pinching the dark gray button on the quick-release with thumb and forefinger **(see Figure 90)**.
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, ensuring that the quick-release clicks in tightly.

Figure 90: Disconnecting GeoMx reagent bottle

> **IMPORTANT:** Use clean gloved hands during this process. Refrain from touching the fittings for bottle S. In addition, ensure that the bottles are securely connected to their fittings (listen and feel for the click) after refilling reagents or emptying waste. A connection that is not clicked in may leak and cause damage to the instrument.

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

EMPTYING THE WASTE

Checking the reagent and waste levels is an important step prior to starting your GeoMx DSP run. The system will check the waste level periodically; if the waste is too full, you will be prompted to empty it. The instrument must be idle in order to empty the waste. You can also click on the **Reagent Status Indicator** in the GeoMx DSP Control Center to initiate the waste emptying process. Follow the prompts in the GeoMx System.

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 quick-release with thumb and forefinger **(see Figure 91)**.

Figure 91: Disconnecting GeoMx DSP waste container

3. Remove the waste bottle from the reagent bay holder **(see Figure 92)**.
4. Unscrew and remove the cap assembly from the waste bottle.
5. Empty the waste bottle.
6. Rinse the waste bottle several times, until no foaming is observed.
7. Screw the cap back on, insert the waste bottle into the reagent bay of the instrument, and reattach the fitting, ensuring that the quick-release clicks in tightly.

Figure 92: Removing GeoMx DSP waste

> **IMPORTANT:** Ensure that the bottles are securely connected to their fittings (listen and feel for the click) after refilling reagents or emptying waste. A connection that is not clicked in may leak and cause damage to the instrument.

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

GeoMx DSP Instrument Maintenance

Maintenance tasks may need to be performed to ensure proper operation of the instrument. These include minor cleaning and related tasks that do not require the presence of a service technician.

System Shut Down

The GeoMx DSP instrument should be shut down and restarted on a weekly basis. Only a user with administrative privileges can initiate the shutdown process. See **DSP system shut down on page 99**.

Cleaning the Slide Holder

Cleaning the slide holder is important to ensure proper instrument and run function. To clean the slide holder after a run:

1. If applicable, remove buffer from loaded slides with a pipette and then remove all slides from the slide holder.
2. Rinse the slide holder thoroughly with diH_2O.
3. Inspect the entire slide holder. Ensure that:
   - The springs of each clamp hinge are clear of all debris.
   - The gasket of each slide area is clear of all debris.

> **IMPORTANT:** Do not remove the gasket from the slide holder. Gasket alignment on the slide holder is extremely important in ensuring a successful run and preventing damage to the GeoMx DSP instrument. Contact geomxsupport@nanostring.com for assistance.

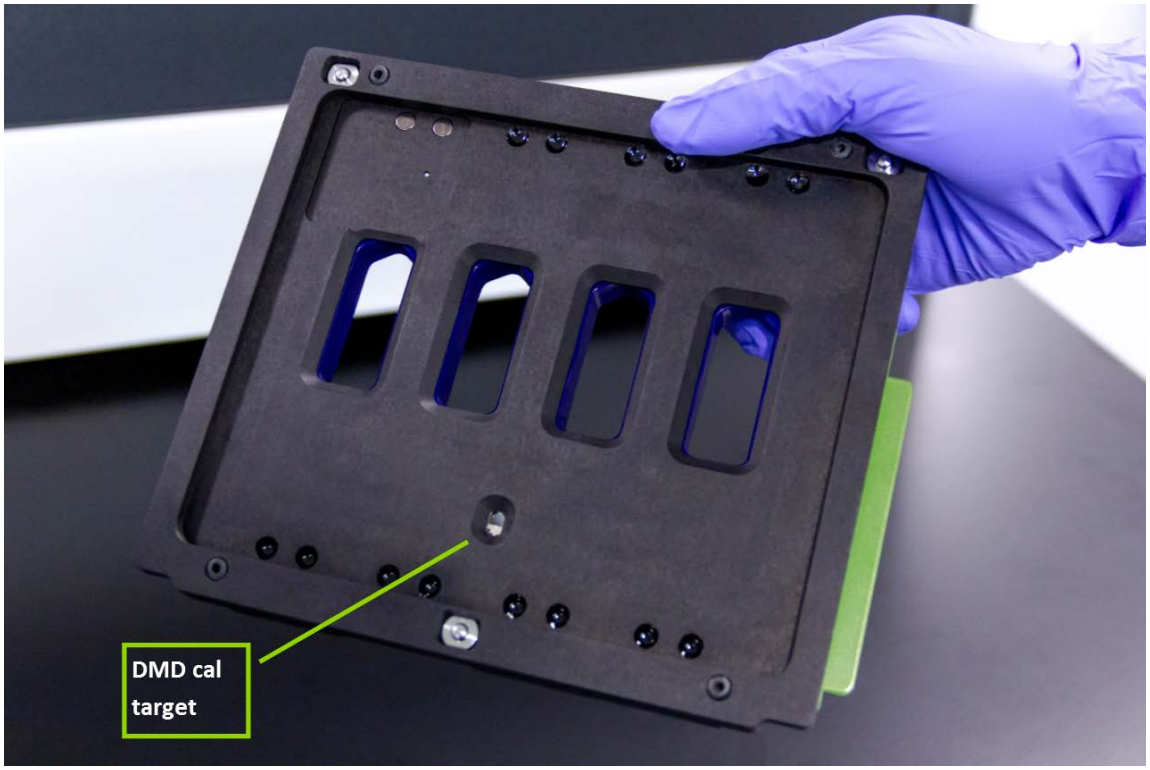


Figure 93: Underside of slide holder with DMD cal target indicated

- Visually inspect the DMD calibration target (see Figure 93) to ensure it is clear of all debris. Do not make contact with this area unless necessary to gently wipe away debris. DO NOT apply pressure to this area. Following this cleaning procedure promptly after each run should be sufficient to keep this area clean.

4. Perform a final rinse with diH_2O.
5. Wipe the barcode on the slide holder clean and allow the slide holder to air dry. Inspect the slide holder again before the next run.

> **IMPORTANT:** If the slide holder has been dropped, its performance could be compromised on future runs. It is recommended in this case to run a test slide before using the slide holder with test samples. Contact geomxsupport@nanostring.com for more specific guidance.

General Cleaning

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

- Always ensure the waste bottle has available capacity before starting a new run.
- If a spill occurs, clean the affected 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

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 NanoString GeoMx Support (geomxsupport@nanostring.com) to request a replacement.

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.

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