SOP#: 017	Date Issued: 8/21/2024	Date Revised: 9/05/2024
TITLE:	**GeoMx Digital Spatial Profiler (DSP)**	
SCOPE:	Research Personnel	
RESPONSIBILITY:	BORC staff	
PURPOSE:	To outline the proper procedures for use and maintenance of the **GeoMx Digital Spatial Profiler (DSP)**.	

1 PURPOSE

This SOP explains how to use **GeoMx Digital Spatial Profiler (DSP**) and scan for a spatially resolved digital profile of analyte abundance.

2 RESPONSIBILITY

It is the responsibility of the BORC staff to ensure that equipment is appropriately cleaned, maintained in good working order, and available for research personnel as requested.

3 BEFORE USE GeoMx DSP

3.1 Slide staining and RNA or Protein slide preparation are done using MAN-10150-05. Prepare slides and incubate biological targets with UV-cleavable probes. Prepare manually or using the BOND RX/RXm Fully Automated IHC/ISH Stainer from Leica Biosystems®.

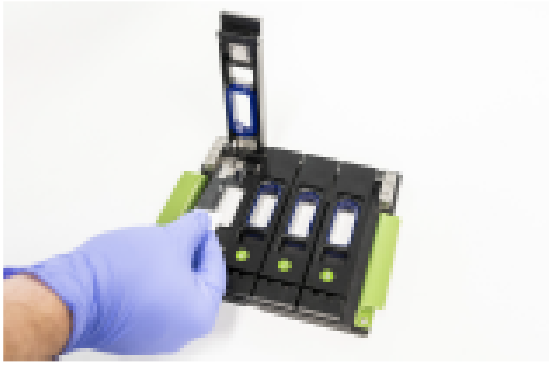
4 PROCEDURES

4.1 **Turn on.** Switch on the instrument at the back of GeoMx DSP and log in.

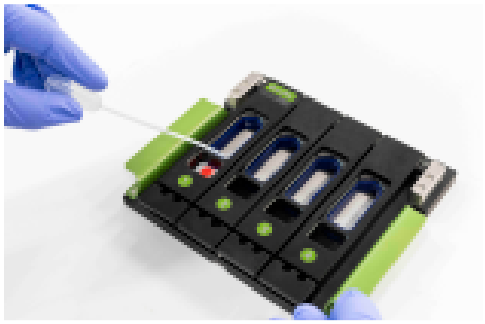
User will be prompted to the software screen for run.

4.2 **Load slides**

Open the slide holder clamp. The slide label should be visible in the rectangular window above the green slide slot number. Tissue may not extend beyond the gasket boundary. Clean the bottom of the slide again, if needed, with a lint-free wipe and 70% ethanol. Lower the slide holder clamp.



4.3 **Add 6 mL Buffer S onto each slide.**



4.4 **Record the location of each slide in the slide holder.**

4.5 **Open the instrument door when prompted. Once the door is open, the system will display the next step**

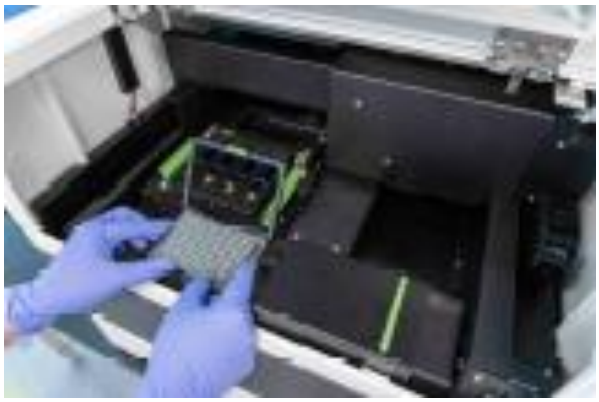
4.6 **Identify Plate**
Each NanoString collection plate has a barcode along one side. This barcode is used to track the plate and its aspirates throughout the DSP workflow. Scan the plate's barcode by holding it at a 45° angle to the right of the scanner and moving the plate between 2-6 inches from the instrument. The barcode will auto-fill when read. Alternatively, enter the barcode manually.
For NGS readout: Select Illumina from the Downstream Counting Platform dropdown field. Choose the Readout Group. This is the selection of plates you want grouped together. Choose from a previously-established group or create a new group.

4.7 **Click Next**

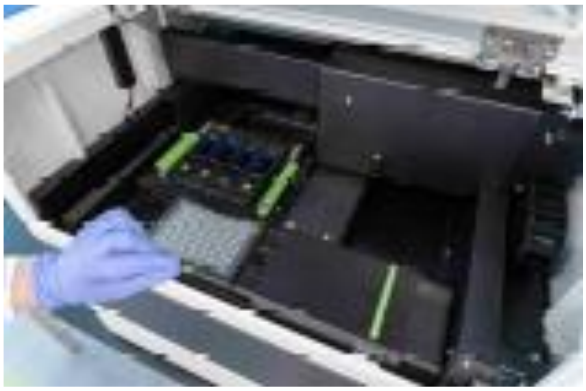
4.8 Insert the Collection Plate

4.8.1 Open the plate holder clamp.

4.8.2 Insert the collection plate onto the plate holder.



4.8.3 Fasten the clamp.



4.8.4 Once the clamp is fastened, the system will display the next step

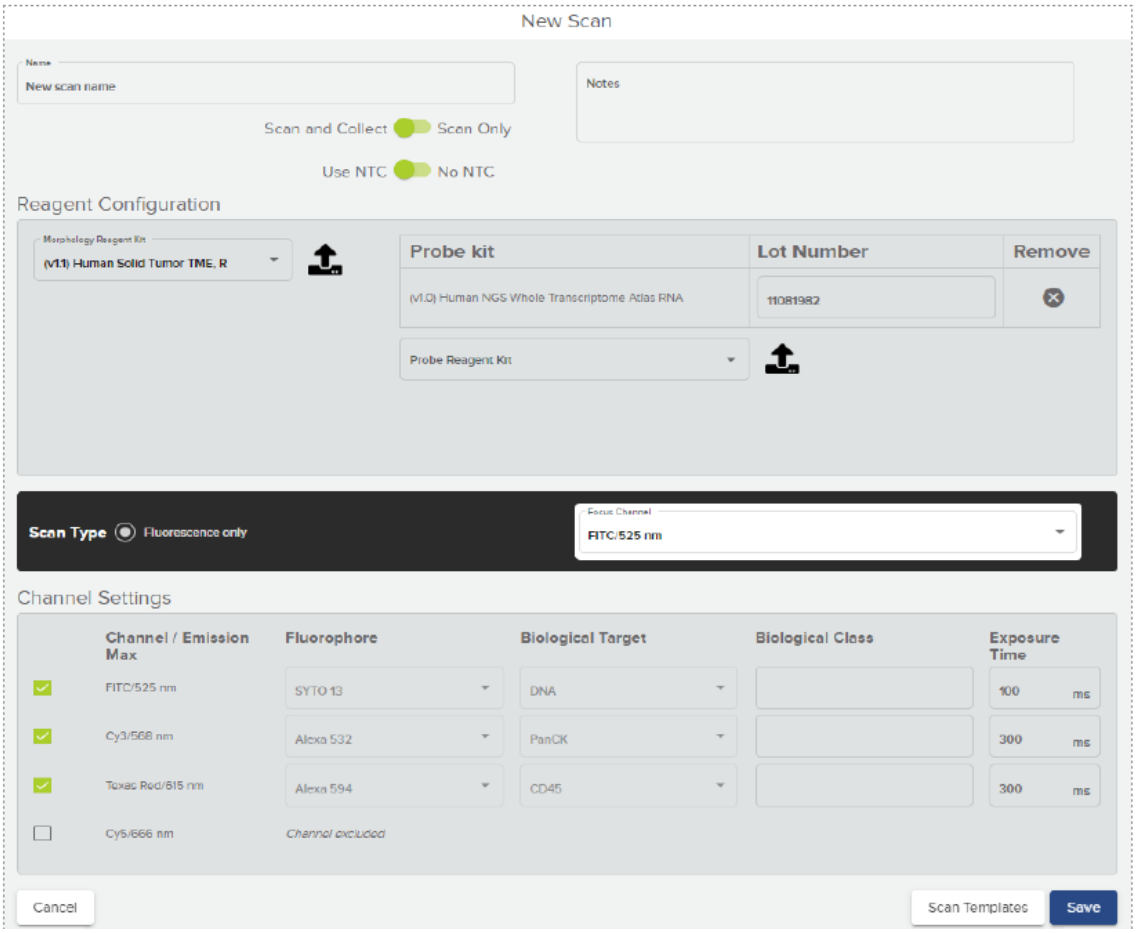
4.8.5 Cover/Uncover the collection plate

4.8.6 Insert the Slide holder

4.8.7 Close the Door. When prompted lower the door to close the door

4.8.8 Identify Slides Once the necessary components for the run have been detected, the system will commence Slide Identification by taking a low-magnification image of each loaded slide

4.8.9 On the desktop screen set scan parameters to Create New Scan



a. Enter a Name for this scan. This should be a unique identifier for this instance of scanning.
b. Toggle to select Scan and Collect or Scan Only. Scan Only mode enables slide scanning and ROI selection without collection.

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

d. Select Reagent Configuration files.GeoMx DSP probe kit configuration (.pkc) files associate GeoMx readout barcodes with RNA and Protein assay targets. Morphology Kit configuration (.mkc) files provide visualization target information to the GeoMx DSP software. Configuration files are not pre-loaded on the instrument and must be transferred using a USB drive. Find the files for the probe kits used on your slides at www.nanostring.com/dspconfigfiles. Once uploaded to the instrument, they are available for selection in the Scan Configuration window.

e. Select the appropriate Scan Type and Focus Channel for your slide. The scan type option will be set to Fluorescence only. The focus channel selected must have abundant signal. FITC/525 nm (default for nuclear stain SYTO 13) is typically used. Verify the Channel Settings fields.

f. Fill in the custom morphology field (if applicable) to populate custom fluorescence exposure settings.

g. If desired, save the scan configuration as a template by clicking Scan Templates, then Save from the dropdown menu. You'll be prompted to name the template.

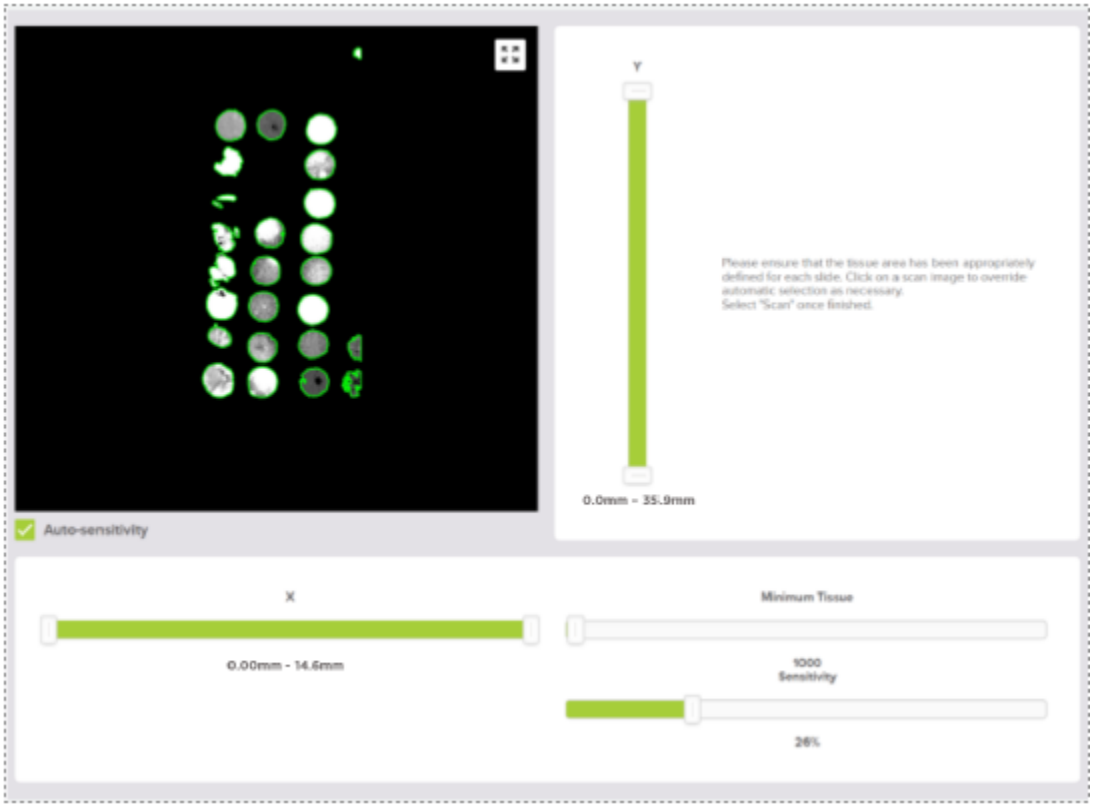
h. Select Save on the Scan Configuration window.

i. Scan Only mode enables slide scanning and ROI selection without collection.

j. Select Next. The system will proceed to the Review Tissue Detection window. Scan Slides. Before the system's 20x scan, it checks the tissue area. You may accept the default parameters automatically detected by the system, or customize them.

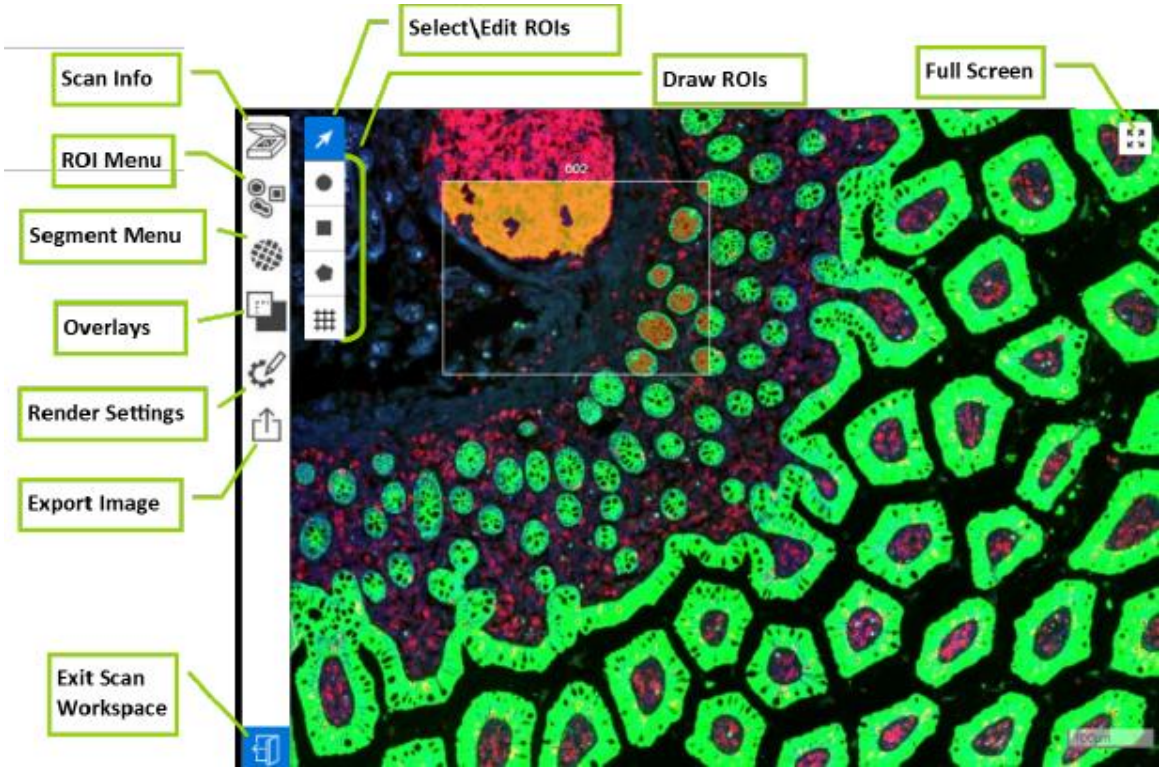
k. Define Scan Area: The scan area result is displayed. Use the tools in this window to select only the tissue on the slide. This minimizes scan time and avoids unnecessary consumption of disk space. Areas within green boundary lines will be scanned. To adjust:

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



l. Select the Scan button when you have completed defining the scan area for all slides. The system will begin a high-magnification scan of each slide, using the defined tissue area and the scan parameters specified in the scan record. **Note**: Scan Only mode enables slide scanning and ROI selection without collection.
    - Once the entire 20x scan is complete for a slide, you may zoom in, zoom out, pan, and switch to full-screen mode within each slide view.
    - Once scanning of a slide is complete, the Edit ROIs button underneath the image will appear.
    - Click the Edit ROIs button to open the Scan Workspace.

m. Select ROIs. Select ROIs. The Scan Workspace allows you to position regions of interest (ROIs) on your scan. If desired, you can also perform segmentation within the Scan Workspace or using an external program such as ImageJ. You can work in the Scan Workspace in Scan Only mode or Scan and Collect mode. In Scan Only mode, scans can be evaluated and ROI selected, but will not be sent for collection. In Scan and Collect mode, following ROI selection and approval, the GeoMx DSP instrument collects tags by exposing each segment of each ROI to UV light and aspirating material from the solution into a well of the collection plate.



4.8.10 Inspect Image

a. Note that red blood cell autofluorescence is very common in FFPE tissues; avoid mistaking red blood cells (which do not contain nuclei) for nucleated cells.

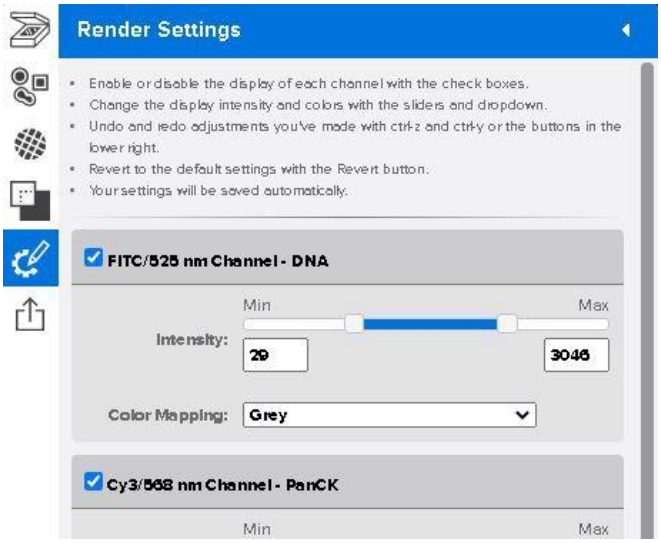
b. Open the Scan Info menu to review scan parameters, date created, and other details.

c. 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

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

d. Open Render Settings Here, you can:

- Change the colors used to represent the different channels on the scan.
- Adjust the intensity of each channel, either with the slider bar or adjusting the values in the editable Min and Max boxes.
- Use the Undo, Redo, or Revert buttons at the bottom of the Render Settings window, if needed.



4.8.11 Draw ROIs

Keep these considerations in mind when drawing ROIs:

a. For protein analysis, the area of an ROI should generally be at least 2,000 µm^2 (approximately equal to a square with side length 45 µm or a circle with diameter 50 µm). ROIs that will be segmented should be larger. As a general guideline, the minimum number of cells per protein ROI (or, if segmenting, per segment) is 20. Open the ROIs List (see page 47) to check the ROI dimensions.

b. For RNA analysis, the area of an ROI should generally be at least 30,000 µm^2 (approximately equal to a square with side length 170 µm or a circle with diameter 200 µm). ROIs that will be segmented should be larger. As a general guideline, the minimum number of cells per RNA ROI (or, if segmenting, per segment) is 100 for NGS readout and 200 for nCounter readout. Open the ROIs List to check the ROI dimensions.

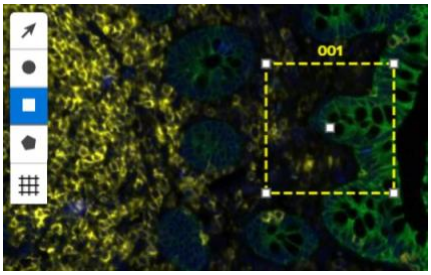
c. Maximum ROI dimensions are 660 µm x 785 µm. A maximum of 380 ROIs can be placed on a single scan.

d. For NGS assays, the instrument designates the first well of each plate, and in addition the first well of any additional readout groups within a plate, as a no-template control (NTC). When planning your ROIs, segments, and plate usage, be sure to account for the NTC well(s). For more information about the NTC, refer to the GeoMx DSP NGS Readout User Manual (MAN-10153).

e. For custom standalone RNA–NGS assays (*without* WTA or CTA), ensure that the ROI selection strategy includes a sufficient number of ROIs of sufficient size, or the resulting NGS library may not contain enough material for accurate library QC. A general guideline for a standalone custom RNA–NGS assay of 20 targets is a minimum collection area of 425,000 µm^2 (equal to 54 circular ROIs of 100 µm diameter), per pooled library. See GeoMx DSP NGS Readout User Manual (MAN-10153) for more information.

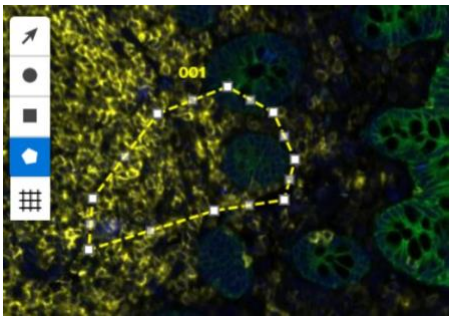
IMPORTANT**: Do not establish ROIs and attempt to collect from areas of scan with poor focus, as this can cause instrument damage.**

Standard Geometric Shapes:



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

Custom Polygon:



a. Select the polygon button from the Scan Workspace
b. Single-click where you would like one vertex of the polygon.
c. Single-click to create more vertices and build the ROI.
d. Click back on the original vertex to complete the ROI. This most recently applied ROI will now have a number designation.
e. Click and drag a vertex to adjust the polygon in shape and size. If resizing causes unexpected changes in polygon shape and size, create a new polygon, then return to the polygon you wish to alter and it should resize properly.
f. Each side of a polygon has a center point; click on a center point to convert it to a vertex. Click on a vertex to convert it to a center point.

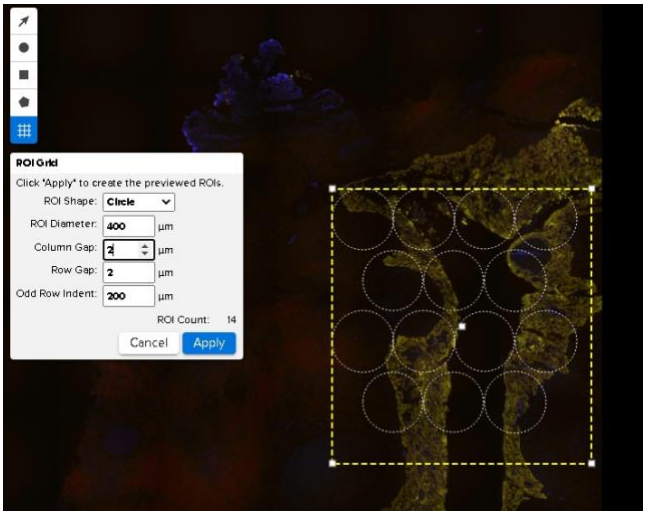
Keep in mind the minimum ROI size and cell number guidelines from the previous page. Placing a square ROI over or near your custom polygon ROI may help estimate its size. Open the ROIs List to check the dimensions of established ROIs.

Grid ROIs:

a. Select the Grid ROIs tool in the Scan Workspace.

b. Select the ROI shape (circle or rectangle), diameter, and column gap (distance between each column of ROIs), row gap (distance between each row of ROIs), and odd row indent (to indent every other row.

c. Click on the scan in the location where you intend to place one corner of the grid and drag. The grid area will appear as a yellow dotted perimeter and the individual ROI previews will appear inside with white dotted line perimeters. The ROI count within the grid will appear in the lower right corner of the grid parameters field.



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

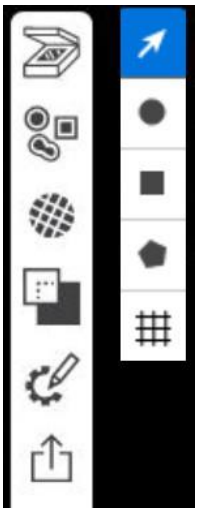
d. When the preview appears as it should, click Apply. The ROIs will be applied to the scan.

e. Once you have placed the ROIs, they become separate ROIs that can be moved and altered individually.

A maximum of 380 ROIs can be placed on a single scan.

Move and Adjust ROIs:

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



a. Click and drag the center point of the shape to move it.

b. Click and drag a white perimeter point to resize the shape.

c. Copy and Paste ROIs by holding down the control button (Ctrl) on the keyboard and clicking and dragging the center point of an existing ROI. Release the click to place the new ROI(s).

d. Select multiple ROIs by pressing Shift and then clicking the center of the desired ROIs.

e. To delete the ROI(s), click the Delete or Backspace button on your keyboard. You can also click the red X in the ROI List (see next step). To delete all ROIs in the scan workspace, select an ROI in the ROI List, then press Ctrl+A, then press Delete or Backspace.

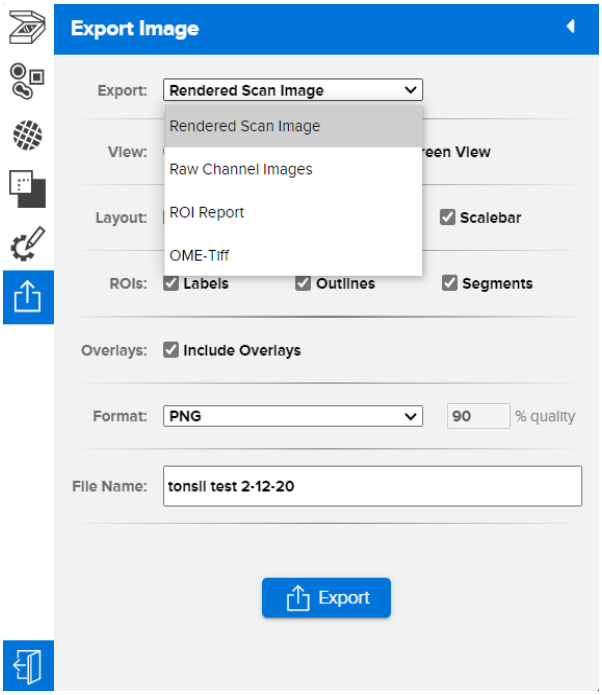
4.8.12 Export Images

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

a. Select the Export menu in the Scan Workspace.

b. The default view is Rendered Scan Image; choose this for a full- color publication-quality image.

- Choose Raw Channel Images to export single- channel, high-resolution images as .tiff files. These may be several GB in size. If size exceeds ImageJ limit, try IrfanView graphic viewer.
- Choose ROI Report for a zipped file with a separate image of every ROI and every segment within that ROI, as well as an HTML summary of those images.
- Choose OME-Tiff to export as a .tiff file containing embedded OME-XML metadata.



c. Use the View, ROIs, and Overlays fields to indicate what to export on the scan

- Rendered Scan Image allows you to choose Full Scan, which exports the entire scan image, or On Screen View, which allows you to manually zoom in and frame the export field as desired.

d. Choose the Format in which you'd like the image exported (.jpg, .png, .tiff).
   - The .jpg format allows you to adjust the file compression in the % quality field.
   - The full exported image will not be zoomed and will reflect the Rendered Scan Image.

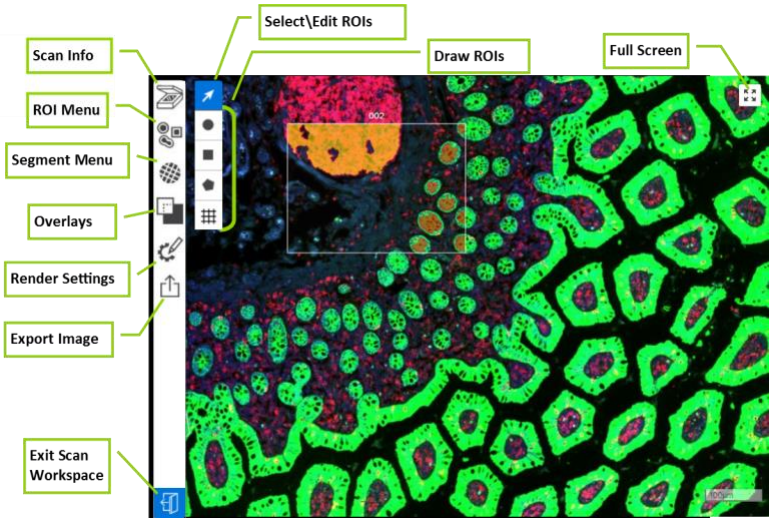
e. Enter the File Name you'd like to use for the image.

f. Select Export.

4.8.13 Exit the Scan Workspace and Approve ROIs for Collection

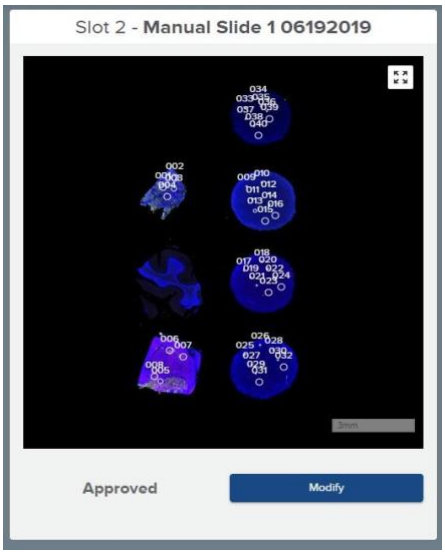
> IMPORTANT: DO NOT attempt to begin collection if the scan is out of focus.

a. Select the Exit button in the lower left of the Scan Workspace toolbar when ROI selection is complete.



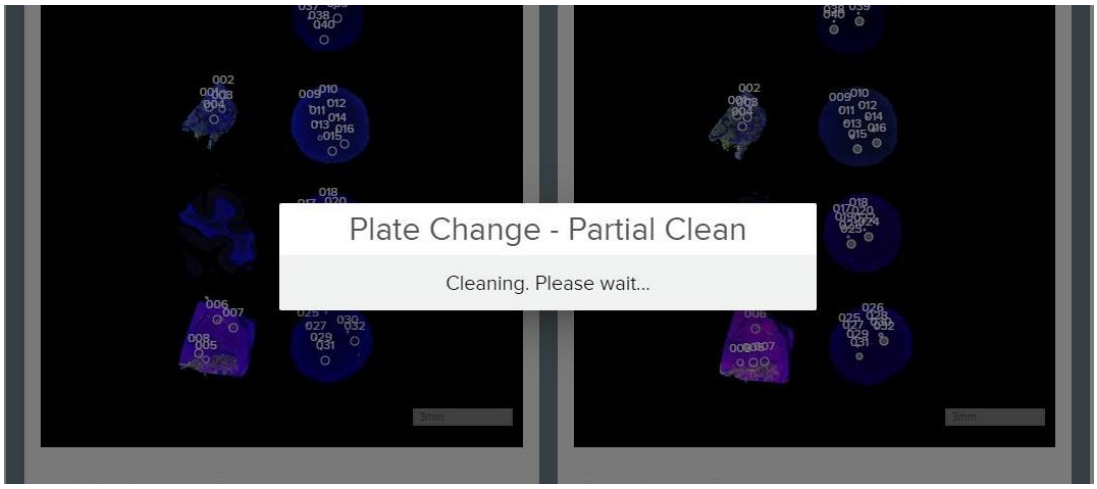
b. If working in Scan Only mode, the Scan Workspace closes and you are returned to the scan gallery.

c. If working in Scan and Collect mode, a pop-up window asks you to Approve ROIs or Save and Exit. The Well Count estimate for your scan appears here (NGS assays must leave room for NTC wells. Approve the ROIs to queue them for collection. Save and Exit to save the ROIs without starting collection. The Scan Workspace closes and you are returned to the slide view.



d. Repeat the steps Select ROIs and Segment ROIs (optional) for each slide. Use the Edit ROIs button to open the Scan Workspace for each scan image. Once ROIs have been saved and approved on a scan image, the button below it will change to Modify. ROIs can be modified up until collection from the slide has begun, then the Modify button will not be available.

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



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

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

4.8.14 Unload Plate and Slides

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

Figure 53: Workflow marker at Collection Complete

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

Removing the collection plate

Follow the on-screen prompts to remove the collection plate from the instrument.

After the collection plate is removed, it can be prepared for readout by NGS

(see GeoMx DSP NGS Readout User Manual (MAN- 10153).

If storing plate before processing for readout, seal plate with adhesive foil to prevent contamination.

Store plate following these guidelines:

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

4.8.15 Remove the Slide Holder

a. Follow the on-screen prompts to remove the slide holder from the stage and move it to a designated laboratory space. Do not close the instrument door yet, as you will return the clean slide holder to the instrument.

b. Using a pipette, remove and dispose of the buffer from each slide in the slide holder.

c. Open each slide slot clamp and unload each slide.

d. Store the slides:
   - Protein slides: Submerge in 1X TBS-T at 4°C, protected from light, for up to 1 day. To store for >1 day, follow mounting procedure below.
   - RNA slides: Submerge in 2X SSC at 4°C protected from light for up to 21 days.

Stability studies indicate that FFPE slides can be stored for 21 days in 2X SSC at 4°C without reducing the number of genes detected in tissue microarray and cell pellet array samples. Morphology marker signal remained functional for the duration of the study. SYTO 13 signal decreased, but remained functional. Nuclear or morphology marker stain can be repeated prior to scanning if deemed necessary.

e. Clean the slide holder and return it to the instrument.

f. Follow the prompts to close the instrument door.

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

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

a. Rinse slide to be mounted with TBS-T or PBS-T. Touch the slide edge to a paper towel to remove excess liquid. Place slides on a flat surface.

b. Using a pipette tip (200 µL tip works well), add one drop (~50 µL) of Fluoromount-G to the slide; add more as necessary to ensure the slide does not dry out and there is adequate tissue coverage.

c. Apply coverslip (align one edge, then slowly lower from one side to the other) and remove excess mounting medium.

d. Allow slide to dry at room temperature in the dark overnight (such as bench drawer).

e. Store slide at 4°C, protected from light, for up to 3 months.

4.8.16 Finalize the Collection Plate

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

a. Plates are finalized by row, not by well, even if some wells in the row are unused.

b. Once you've finalized a set of rows in a readout group, you cannot add more unfinalized rows to that readout group. If you intend to add additional aspirates to a collection plate, do not finalize the readout group until all aspirates have been collected.

c. You can later re-insert the plate and use the unused rows:

- If the previous collection has been finalized, a different readout group must be used. Collection will begin in the next available row of the plate.
- If the previous collection has not been finalized, the same readout group must be used. Collection will begin in the next available well of the plate, unless the new collection is with a different kit configuration, in which case it will begin in the next available row.
- Some combinations of assays are not allowed in one plate. If an incompatible combination is attempted, the instrument will prompt for a new plate.

d. You can return to this window to access the readout files by clicking the plate icon and entering the plate number or searching by readout group name.

For NGS readout:

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

For nCounter readout:

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

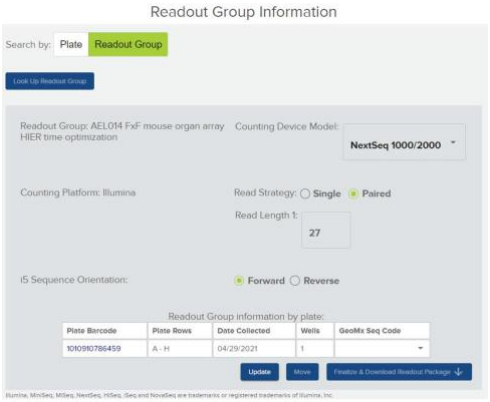
e. To finalize a plate, click on the plate icon area at the lower right of the GeoMx DSP Control Center. If the plate is not currently in the instrument, enter its barcode.

f. After collection, the wells of the plate icon in the lower right of the Control Center should appear green for each collected well. Refresh (F5) to update the display. After Finalizing the plate, the entire row should appear green.

g. The Plate Information or Finalize Plate window will appear:

For NGS readout:

The Plate Information window provides a table summarizing the status of each row of the collection plate.



1. Click Readout Group to see a list of available readout groups.
2. Select the appropriate Illumina platform from the Counting Device Model menu.
3. Choose Single or Paired Read.
4. Enter read length 27 into the Read Length field.
5. Choose the correct i5 Sequence Orientation.
6. Establish which GeoMx Seq Code or Pro Code plate should be associated with the Plate Barcode in the Readout Group Information grid.
7. Click the Update button.
8. Select the Finalize and Download Readout Package button.

For nCounter readout:

The Finalize Plate window provides a table summarizing the status of each row of the collection plate.

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

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

For NGS readout:
continued

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

10. Insert a USB drive into the instrument.

11. Click OK in the Readout Package Successfully Created window to save these files to the USB:
• Lab Worksheet contains information on the contents of each well of each plate, ROI coordinates, nuclei count, recommended sequencing depth, and more.
• Configuration File will be used by the GeoMx NGS Pipeline software to convert the Illumina FASTQ files into DCC files.

• Seq Code or Pro Code UDI Indices file is needed for your NGS Illumina platform run except when using the NextSeq 1000/2000.
• For NextSeq 1000/2000 users, a samplesheet.csv and whitelist.txt replace the Seq Code UDI Indices file.

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

For nCounter readout:
continued

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

4. Select the Finalize button.

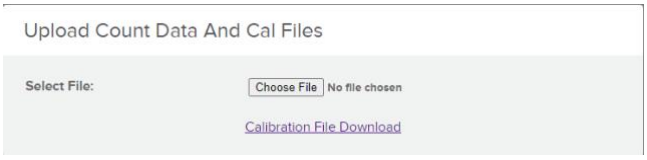
5. Insert a USB drive into the instrument.

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

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

4.8.17 Uploading counts

a. Click on Data Collection then Upload Counts/Cal Files. The Upload Count Data and Cal Files window opens.



b. Click Choose File and navigate to the zipped counts folder: DCC.zip or output.tar.gz for NGS readout, or RCC.zip for nCounter readout.

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

For NGS readout:

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

For nCounter readout:

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

5 MAINTENANCE

The **GeoMx Digital Spatial Profiler (DSP**) should always be left clean and dry whenever the system is not in use. The software should be shut down and the **GeoMx Digital Spatial Profiler (DSP**) power switch should be turned off.

After use, the system should be flushed through with sterile water to remove all traces of sample from the tubing and optical surfaces. If using diluent with a high concentration of dissolved solids (i.e. saline solution), flush clean water through the system after use.

6 REFERENCES

Refer to the manufacturer's manual for additional information.

END OF DOCUMENT