GeoMx® DSP Operation Guide for Service Providers

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NanoString Technologies, Inc.

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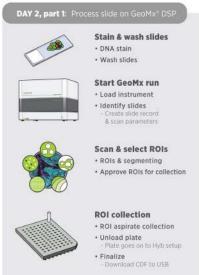FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.

GeoMx DSP Introduction

GeoMx® Digital Spatial Profiler (DSP) is a new technology platform which enables spatial analysis of RNA and protein in tissue specimens on slides. Samples are stained with large panels of pre-mixed biological probes (each which incorporate a unique, UV-cleavable DNA barcode) and fluorescently-labeled morphology markers (used to visually or computationally elucidate the morphology of the tissue). Using the fluorescent morphology markers for guidance, defined regions of interest (ROIs) are illuminated with UV light and the cleaved barcodes from these ROIs are then counted on an nCounter platform. This allows for high-plex RNA or protein quantitation from spatially-resolved regions within the tissue. The resulting counts constitute an expression or protein profile of key targets across that ROI and elucidate the biology specific to that region.

GeoMx DSP In a Service Environment

In **FIGURE 1** the GeoMx DSP Practical workflow is summarized (more details in the GeoMx DSP Instrument User Manual). When running GeoMx DSP experiments in a service environment client input is recommended and may sometimes even be required at some points in this workflow. Because of the way the GeoMx DSP instrument and workflow is setup, this interaction does not need to be physically on-site, but can be done remotely. Points of remote interaction are illustrated in **FIGURE 2** indicating the practical workflow. Potential points of customer/client input can be at the stage of ROI selection and segmentation and at the stage of data analysis and interpretation (steps 3, 6, and 7 in **FIGURE 2**). In the subsequent sections, some suggestions are made, and guidelines are given in order to have a streamlined workflow with an optimal customer or client interaction where needed.
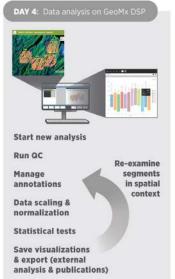

FIGURE 1: GeoMx DSP Overall Workflow

FIGURE 2: GeoMx DSP Practical Workflow

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External Access to the GeoMx DSP Software

There are a number of ways in which remote access to the GeoMx DSP software can be organized (summary in **TABLE 1**). This access can be setup to be: 1. visual access only, where the second party (i.e. the customer or client) can only view the GeoMx DSP software environment but the software control is driven from the host-side (i.e. CRO or Core/Service Facility), or 2. the second party also gets control over the DSP software (either full control, or limited control, depending on the way it is setup).

The easiest way to set this up is via online-meeting platforms (i.e. Zoom or Cisco WebEx). These platforms allow both "view only" modes (desktop sharing), but also have the capability of handing over desktop control. For example, the service provider could setup an online meeting with a client to discuss/review the ROI selection strategy. If desired, during this meeting the service provider could give the client desktop-control access to control the DSP instrument (from the service provider's desktop environment while connected to the GeoMx DSP software through the Google Chrome-based web app, via the service provider's internal network).

Alternatively, the service provider can setup a Virtual Private Network (VPN) connection for their client. This will allow the client to remotely connect to the internal network of the service provider, allowing for a direct internal network connection to the GeoMx DSP software. User, group, and folder access permission can be put in place to ensure a secure environment for each client to ensure data protection and exclusivity. Via this VPN connection, access to the GeoMx DSP software is enabled and the client can do their own ROI selection and, ultimately, data analysis. Important to know is that such data connection would be similar to remote connection to the GeoMx DSP instrument from the physical internal network of the service provider, with the same capabilities and restrictions. One of these restrictions is that such a connection will not allow for the operation of the GeoMx DSP instrument itself; there will only be access to the Data Analysis Suite, but not to the Instrument Control Center.

	Online Meeting Environment	VPN
Simultaneous Logons	1	Multiple
ROI selection and segmentation	Yes, driven by service provider and guided by client	Yes, driven by client
Data Analysis	Yes, driven by service provider and guided by client	Yes, driven by client
Folder visibility/access permission	No, controlled by service provider during online session	Yes, User-Group-Folder access control
Simultaneous instrument operation	Yes	Yes
Number of clients simultaneously connected	>1 Note: too many simultaneous connections may impact instrument performance in terms of slide processing time. It is recommended to schedule and keep track of logons in a calendar.	>1 Note: too many simultaneous connections may impact instrument performance in terms of slide processing time. It is recommended to schedule and keep track of logons in a calendar.

TABLE 1: overview of remote access options

Setup online-meeting environment

For setting up online-meetings using for example Cisco WebEx, Zoom, or Microsoft Skype (or alternative solutions) please consult the applicable provider's support and guidance documentation.

Setup VPN access environment

For on-demand access to the GeoMx DSP software remotely, it is recommended to require a VPN connection from clients with limited access to the web service on the instrument. An SSL VPN solution such as Pulse Connect Secure or Cisco SSL VPN will allow clients to securely connect to the instrument's web interface to perform ROI selection and data analysis. The VPN can log all incoming connections to provide an audit trail. In this environment, VPN access does need to be managed separately from the on-instrument access control. The service provider should work closely with its IT and Network Administration teams to design a secure solution.

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User-Group-Folder Access

Generating users, groups, and folders with the right permissions will help protect client data from being visible and accessible by other clients when for example logged on to the instrument through a VPN connection and logged on to the GeoMx DSP Data Analysis Suite. See the GeoMx DSP Instrument User Manual for detailed guidelines. In summary, Folders can hold slide records, scan cards, and data analyses. The access model is based around groups, where folder access is given to one or more groups. There are two permission levels (i.e. roles) with respect to users: *admin* or regular *user*. *Admin* users have access to everything and can also create new users and groups. A regular *user* only has access to folders that the group/groups to which they belong.

A proposed way of working is to:

1. Create one folder for each client, naming using whatever convention is appropriate for the service environment, i.e. client name or anonymous identifier
1. With the client folder, create sub-folders representing different projects
1. Set group access permissions to these folders (main client folder or sub-folders)
1. Create users and assign each user to groups linked only to folders to which they need access

To illustrate the above, in Figure 3 an example of a folder structure and their group assignments is displayed. A user in the **Ph1-G** group would see and be able to access everything in folders "Pharma 1", "P1-ProjectA", and "P1-projectB", but would not see and not have access to the "BioTech 1" folder and its sub-folders. A user in the **BT1-G-Main** group would see and be able to access everything in folder "BioTech 1", "BT-1-Project A", and "BT1-ProjectB". A user in the **BT1-G-Main** group would also see the "Pharma 1" folder but would not be able to see the contents or access anything in that folder. A user in the **BT1-G-Main** group would see and be able to access all content in the "P1-ProjectB folder.

Creating Slide Records

Slide records can be created during the DSP run workflow. To ensure a faster workflow, new slide-records can already be set as a pre-work step by the service provider's technician or project manager. For more details, see the "Creating Slide Records" section of the GeoMx DSP Instrument Manual.
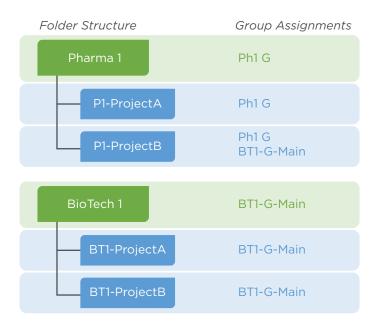

Folder Structure   *Group Assignments*

Pharma 1	Ph1 G
P1-ProjectA	Ph1 G
P1-ProjectB	Ph1 G / BT1-G-Main
BioTech 1	BT1-G-Main
BT1-ProjectA	BT1-G-Main
BT1-ProjectB	BT1-G-Main

FIGURE 3: example of folder structure and group assignments – folder names are user-chosen and can be de-identified to protect customer identity if necessary

Study Design and ROI Selection in a Service Environment

Completing a study for a client in a service environment can be streamlined by making sure the experimental aims of the study are discussed and documented (see DSP Project Workplan document) well in advance with all parties involved in the project. Tightly connected with the experimental aims are *ROI selection and segmentation strategy*, which in turn guides appropriate *morphology markers* needed. Please refer to MAN-10108 "GeoMx DSP Experimental Design Guideline" for basic best practices around experimental setup. In addition, we have found the following practices to be useful as a service provider through the Nanostring Technology Access Program.

Discuss Morphology Markers and ROI Selection Strategy in Advance

Morphology markers, in the context of GeoMx DSP, are any fluorescently-conjugated reagents used to visualize the tissue. While Nanostring has several pre-kitted morphology markers for solid tumor/tumor microenvironment visualization, Alzheimer's disease, and Parkinson's disease, the customer is free to use whatever antibodies they wish provided they are directly conjugated to an appropriate fluorophore. Wavelength specifications for the filters available on the instrument can be found in MAN-10088 GeoMx DSP Instrument Manual, but in general terms they are FITC, Cy3, Texas Red, and Cy5.

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Many companies provide antibodies directly conjugated to these fluorophores, or there are commercial kits available for conjugating antibodies yourself. Please note, we recommend use of Syto 13 or Syto 83 as a nuclei marker, which occupy the FITC or Cy3 channel, respectively. Nuclei staining is the most straightforward target for autofocus on the instrument and is strongly recommended for all experiments. Use of DAPI or other UV-excitable nuclear markers is not compatible with the GeoMx DSP. Outside of our pre-kitted morphology reagents, antibody titration may be required to ensure sufficient staining for visualization, however the concentrations and exposure times listed in the manuals for our off the shelf reagents are good starting places.

Use of RNAscope reagents, or fluorescently-conjugated *in situ* hybridization probes, is compatible with the GeoMx DSP RNA assays. Refer to MAN-10087 Slide Preparation for a protocol. This can be additionally beneficial with targets that do not have viable antibodies generated to detect them, or for identifying cells that have active transcription (i.e. using RNA scope to identify transgene producing T-cells in the context of immunotherapy, or virally infected cells). Please note, at the moment, RNAscope signal cannot always be used for ROI segmentation due to the punctate, intracellular nature of the staining pattern, but can help with guiding the placement of the ROIs on the tissue.

Unless the tissues of interest have been extensively characterized by Immuno-Fluorescence (IF) in the past, we strongly recommend performing a *fluorescent morphology marker only* stain of the tissue as a pilot experiment. This will allow the validation of morphology marker(s) performance and enable the parties involved in ROI selection (i.e. client, GeoMx technician, Pathologist, etc.) to discuss the results and refine their experimental approach. This will 1) ensure that the ROI selection strategy proposed by the client is feasible in the tissues they intend to have analyzed, and 2) allow for a detailed scope of work and ROI selection criteria to be generated, which will streamline the process for the larger experiment. Once the slides are on the instrument, they must remain on the instrument through collection, within 6 hours. Having a robust, predefined ROI selection strategy minimizes the time required for those approving the ROIs to be available and either in front of the instrument or remotely engaging with it. These meetings can be arranged via teleconference environment or VPN access to allow the client to interact with the software directly (see **TABLE 1**). This initial fluorescent marker evaluation will be especially important in the context of GeoMx RNA applications; not all antibodies will perform ideally under the antigen retrieval conditions of the RNA assay, and some optimization may be required.

Addressing and Validating Custom Oligo-tagged Profiling Content

If a customer is interested in custom oligo labelling of antibodies or ISH probes, reach out to GeoMXsupport@nanostring.com or to your Nanostring sales representative. Depending on the service environment (i.e. a Contract Research Organization), the customer *may* contract directly with the service provider for these services and list this service as a pass-through cost. For custom RNA reagents, probes can be designed with very similar algorithms to the ones that are utilized for NanoString's nCounter® bulk gene expression assays tiling across a target transcript. NanoString does not do any target testing for custom probe validation, but the end user is free to (and should) validate them with experimental positive and negative control samples.

For antibody labelling, our partnership with AbCam allows customers access to the AbCam library of antibodies validated for use in IHC. AbCam can label up to 5 antibodies for inclusion with any Nanostring protein core assay (i.e. the Immune Cell Profiling core or Neural Cell profiling core). The labelling service through AbCam will include target testing pre- and post-oligo conjugation. If the customer is interested in an antibody not available through AbCam, or an antibody they generated themselves, antibodies can be conjugated through the Nanostring Protein Barcoding Service (PBS), up to 10 antibodies total. Contact your NanoString sales representative for more information. Validation by NanoString of PBS custom conjugated antibodies is not possible because of limited availabilities of different tissue types. NanoString recommends the end user to validate pre and post conjugated antibodies on positive and negative tissues, or discuss the possibility with NanoString's PBS to perform some validation if positive and negative tissue samples can be provided by the end-user. As a guide, please refer to Merritt et al., Multiplex digital spatial profiling of proteins and RNA in fixed tissue, *Nature Biotechnology*, 38, pages 586-599 (2020), and our white paper "Multi-step Antibody Validation for the GeoMx Digital Spatial Profiler."

Batching Slides to Minimize Variability

Slides that were prepared for the protein assays are stable, when mounted in Fluoromount G, at 4°C in the dark for up to 4 weeks. In a service environment, this allows for flexibility in batching work according to the demands of the project queue and staffing, as well as minimizing any potential variability due to different days/times/technicians involved with slide preparation. For the RNA assay, once labelled, slides may be stored in 2X SSC for up to 48 hours at 4°C in the dark before they must be processed through the GeoMx DSP instrument to avoid loss of signal.

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Data Transfer Guidelines

At this moment, the GeoMx DSP software does not have an import/export functionality allowing to export full studies and import them in another instance of the GeoMX DSP software.

The following data types may be exported and transferred to the service provider's client, with or without an accompanying descriptive report.

Datasets

Datasets may be exported in .xlsx format by opening or creating a data analysis study, going to the Datasets Panel, and hovering over a dataset name followed by clicking the **export** icon. More details can be found in MAN-10090 GeoMx DSP Data Analysis manual.

As a minimum it is recommended to provide the *Initial Data* and the *QC Data* (representing the raw data) to the client, as from here all subsequent data processing and analysis steps can be done manually outside of the DSP software. Please contact GeoMXsupport@nanostring.com for sample data sets if needed.

Images

Slide scan images may be exported from the Scan Workspace environment in the Export menu. Rendered Scan Images can be exported as a full-color publication quality image. Images will be exported in PNG format and one can choose to export the *scan only*, the *scan + segments*, the *scan label + segments*, or *everything*. Alternatively, RAW Channel Images can be exported in case single-channel high-resolution images are required. Depending on the chosen image export type, images can be exported as .jpeg, .png, or .tiff. A more detailed description and more information can be found in MAN-10088GeoMx DSP Instrument Manual.

ROI images

From the ROI menu in the Scan Workspace, for each ROI individually the Export Image icon can be clicked in order to export a 16-bit multi-channel tiff image. Alternatively, there is also an option to export all ROI images at once.

These exported images can be used in an external program (such as ImageJ) to create segments within an ROI outside of the GeoMx DSP software. Subsequently, the generated masks can be imported back into the GeoMx DSP software to apply the masks on the ROIs.

In order to generate a color RGB image from an exported ROI image, a program like *ImageJ* (https://imagej.nih.gov/ij/) or alternative can be used. RGB images can be more easily used for reporting purposes (e.g. in a PowerPoint presentation or in a written project report). In *ImageJ*, the steps to take are:

- Open multi-channel tiff in *ImageJ*
- You will now have an image that consists of multiple channels
- To switch the view between the channels, scroll to the desired channel image with the horizontal scroll bar (or use the ">" and "<" keys)
- In the menu go to **Image > Color > Make Composite**
- Click OK to confirm
- Optional: in order to change the color of each channel:
  - Scroll to the desired channel image with the horizontal scroll bar (or use the ">" and "<" keys)
  - In the menu go to **Image > Lookup Tables >** pick the desired color for the selected channel image
  - Repeat for the other channel images
- In the menu go to **Image > Type > RGB Color**
- In the menu go to **File > Save As >** save the image in the desired file format

Visualizations

Visualizations from datasets may be exported using the exports functionality in the visualizations pane. Click the Export (.xlsx) icon in order to export the data on which the visualization at hand is based on. Click the Export(.svg) icon in order to export the visualization image to an .svg image file. More details on Saving & Exporting of data and images can be found in the GeoMx DSP Data Analysis manual.

For more information, please visit nanostring.com

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