GeoMx® DSP Software v2.1 Tips & Tricks

This document accompanies the GeoMx DSP Instrument and Data Analysis manuals for software v2.1.

Top 7 Tips & Tricks

1. In the event of a failed run and with the advice of GeoMx support, it is possible to restart the workflow. To do so, click the **Assist** wrench icon (Figure 1) and select **Restart Data Collection Workflow.**

    - **Ensure that the instrument door is closed before restarting data collection.**

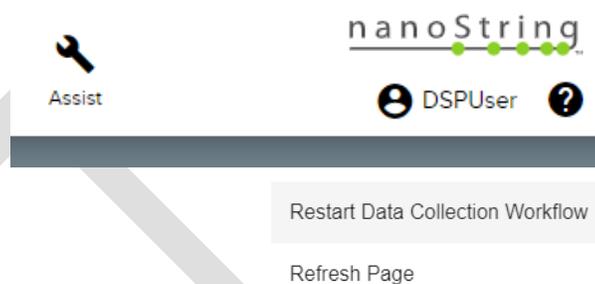   - If a blank screen appears, refresh the page (see below).

Figure 1: Assist wrench icon to Restart Data Collection Workflow and Refresh Page.

2. If the screen appears **blank, inaccurate, or unresponsive, refresh the page.** This may be performed remotely or directly at the instrument. To do this, use keyboard shortcuts **Ctrl+R** or **F5** or click the **Assist** wrench icon (Figure 1) and select **Refresh Page**. You may also use Ctrl/Alt/Delete to log out of and then back into Kiosk mode.

3. **Shutdown and restart the GeoMx DSP instrument <u>on a weekly basis.</u>**
    - When first opening the GeoMx software after rebooting, click on **Assist** wrench icon (see Figure 1) **> Restart Data Collection Workflow.**

4. When downloading logs, **log in to the instrument directly** rather than remotely. Log files may take several minutes to download; the increased time incurred when remotely connected may result in the system timing out and interrupting the download.

5. **DO NOT open the reagent bay door during Collection.**

    - **Opening the reagent bay door during a run may cause the run to pause.** After closing the reagent bay door, you may need to manually restart data collection workflow (see above).

6. After finalizing the plate and **downloading the CDF and worksheet, DO NOT select Show All from the downloads bar.** This will result in kiosk mode locking. In this case, contact geomxsupport@nanostring.com.

7. **To prevent a critical error, leave the slide tray in the instrument** unless you are loading/unloading it or cleaning/drying it. **Ensure it is in the instrument before shutting down or restarting.** If the door is locked, use the Assist button > Restart Data Collection Workflow to reach the prompt to open the door.

General

- Inadvertently running the GeoMx instrument with a **bottle disconnected** may result in **multiple fluidics error messages**. If the error is not resolved after reconnecting the bottle, there may be air in the lines. In this case, contact geomxsupport@nanostring.com.

- **Do not** attempt to **shut down** the GeoMx DSP instrument while a run is in progress or when either door is open.

- If the GeoMx DSP **Control Center header is missing** when in kiosk mode, logout of windows using ctrl-alt-del, then login again as *Kiosk User.*

- After changing reagent bottles and closing the reagent bay door, the instrument may erroneously display a **Plate Change - Close Door** message. Click **Next**; this will complete the bottle change process.

- An error may appear while restarting the GeoMx DSP after making a change in **File Management** under **Administration**. If this appears, shutdown and restart the GeoMx instrument as instructed on page 1.

Figure 2: Restart error

- When remotely connected, closing the Chrome browser will automatically log you out of the GeoMx system.

- When logging into the GeoMx system, the **Cancel** button does not cancel the login process as expected. When logged in directly, use Ctrl Alt Del and then log out, then log back in. If remotely connected, close the browser and reopen.

- **Runs that are in-progress while DCC files are being uploaded** to the system may experience lag times or failure. To avoid this, do not attempt to load DCC files from more than two NGS plates at a time while a run is in progress.

- The **Scan Preview** in the GeoMx DSP Control Center may **disappear** after **editing Notes**. The Scan Preview may not load if you **click on one preview** before all have loaded. View each scan by opening in Scan Workspace.

- Chrome does not connect to DSP on a Mac. To manually connect, while on the Chrome error page, type 'thisisunsafe'. There is no text box, just make sure to click anywhere on the error page and then type. DSP will auto load.

- Do not click on e-mail or website links in the GeoMx Help documentation while in kiosk mode. If inadvertently clicked, use Ctrl+Tab to return to GeoMx.

- When exporting an image, the progress dialog may appear stalled at 99%. The export may still be in progress; this dialog will disappear when the export is complete. Large files may take hours to export.
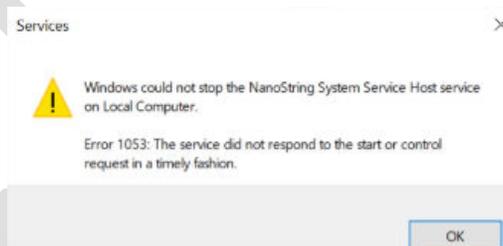
Admin

- **Do not use spaces in usernames.** Usernames with spaces may not be able to view backups in the archive.

- Do not enter a phone number when creating a user, as this may cause an error, preventing you from saving the new user.

- **To download a specific day's logfile,** select a **Custom Range** that extends at least one day before and one day after the day desired.

- If deleting a group, that groups' folders can be transferred to the **All Users** group. Right-click on the folder of interest and select **Edit**; the **All Users** group should be selected. Click **Save** to make this change go into effect.

- When local storage is full, the system provides an estimate of the space needed in GB, however the dialog may read "MB".

- In order to change a user's role (Admin to User, for example), that user must be assigned to no more than one group.

- A critical error state or cascading errors may cause slowness or unresponsiveness in the UI. If no response is observed for >30 minutes, please contact geomxsupport@nanostring.com.

- In the Network window, you will see the **Machine Name** and the **IP address** (see Figure 3). If not, restart the system: shut down from the menu, switch off the switch in the back, then after 10-20 seconds, switch it back on. Do not attempt to turn on Archive Settings while a run is in progress.

- If Archiving is working, a file *mountverification.txt* will be written in the destination folder almost immediately. Then, initial backup of the GeoMx DSP files can take up to 2 days to complete. **To troubleshoot archiving**:
  - Ensure that your GeoMx software is v1.4.2 or later, and your institute network is set up for SMB3 file sharing.
  - Enter the Remote Server path as \\123.45.6.78\\FolderName using the server IP address and your designated folder. 4 opening backslashes may be needed.
  - Try the username in different formats, such as *username*, *username@domain*, or *username@domain.edu*
  - Do not use these characters in your password: $! /
  - Confirm that the credentials to access the folder have *reading* and *writing* permission.
  - Nested folders may be an issue – try to set the Archive folder in the root of the server as shown in above example.
  - A network load balancer may be an issue – contact GeoMx Support to discuss workarounds.

Figure 3: Network Settings window

Run Setup

- The system needs to be in an **idle state** (see page 9) in order to be able to change reagents. The reagent bottle status indicators are managed through the bottle change wizard. **Click on the Reagent Status Indicator in the Control Center and follow the wizard steps to change a bottle or empty the waste bottle.** DO NOT top off any reagents or pour/pipette reagents out of their bottles.

- **Deviating from the GeoMx loading steps by failing to insert slide holder** during the appropriate step or by loading the collection plate before the UI instructs may result in an error or a stalled UI. In these cases, **restart the workflow** (see page 1).

- When **creating a new slide under Records,** if the **Save slide** button is disabled, click the **Cancel** button and create a new slide.

- **When creating scan parameters, failure to select a Core Kit will result in Scan-only mode (no aspirate collection).**

- The best practice for **uploading a config file** is to upload it prior to starting the run from the **Kit Management** section under **Administration**. If encountering an error uploading a config file or if not seeing a newly-uploaded config file in the dropdown when creating a slide record:
  - Ensure that the file you are uploading has been unzipped.
  - Refresh the page (see page 1) and use the same config file.
  - Alternatively (or in addition, if the action above was not successful) you may cancel the creation of the slide record and start again.

- Once a **Core kit** is selected in the Scan Parameters window, only **module kits** compatible with that core kit will be shown in the probe kit dropdown.

- If doing a **plate swap** for your **NGS platform** run, do not use a plate that was previously partially-used for **nCounter platform** aspirates. The system will initially accept the plate but will then repeatedly prompt you to swap the plate again.

- If your slides are not detected by the instrument, check that there is no tissue extending beyond the allowable tissue area (see green area in Figure 4). Scrape parts of the tissue with a clean blade if necessary.

- Plate change is limited to 8 plates at this time. Do not plan runs that will result in more than 8 plates.
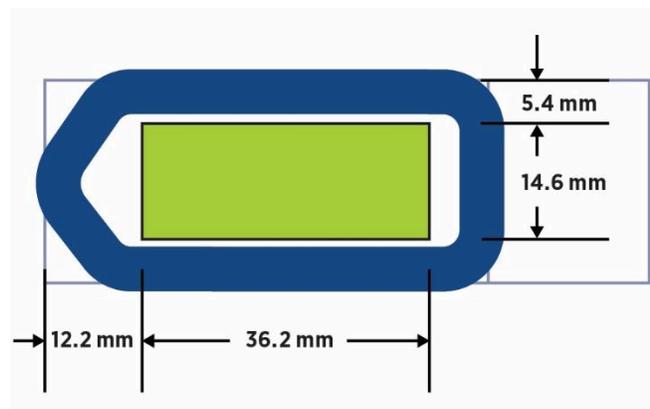

Figure 4: Tissue area boundary on slide

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ROIs and Segments

- When **copying and pasting existing ROIs** in the Scan Workspace, the GeoMx **system does not prevent against overlapping ROIs.** Inspect ROIs to ensure none are overlapping; delete any that overlap.

- **Do not attempt to copy and paste an ROI after it has been segmented**.

- **An error may appear if copying and pasting ROIs too rapidly or if deleting ROIs too rapidly**. If encountering an error when attempting to copy/paste, move, or delete an ROI, wait a moment and try the action again.

- **Do not rename an ROI after it has been moved** (without first saving it in its new location); doing so results in the ROI moving back to its original location.

- If viewing a segmented ROI and then clicking on another ROI in the ROI list, the view may jump to the other ROI but the segment masks of the previously displayed ROIs may still be displayed on the screen. Using the mouse, zoom out just a little bit and the old segment mask will disappear and the new one will be displayed correctly.

- **Resizing the most recently touched polygon** may cause **unexpected changes** in the polygon size and shape. Create a new polygon, then return to the polygon you wish to alter and it should resize properly.

- An error may appear when creating or removing vertices from a polygon, even though the action was successful. Dismiss the error and continue.

- Leave the **Override All** values at the bottom of the **segmentation window set to 0**. Change an **Override All** value only if you want to override all of the ROI thresholds for that channel.

- If exiting Scan Workspace without approving ROIs, slide status may read *Scanned* instead of *Annotated*.

- Channel settings should be modified after scanning *only if* a re-scan is planned. The **Scan Preview** in the GeoMx DSP Control Center may disappear. View each scan by opening in Scan Workspace.

- Names for segments imported from an external masking program will be truncated at 100 characters, potentially resulting in non-unique names. Using names greater than 175 characters in auto-segmentation within the Scan Workspace may result in no segmentation.

- The collection order, well order, and name order of segments may differ from each other, as the system optimizes aspirate collection to reduce complexity.

- **Changing a Channel Thresholds setting to 0** on an ROI will revert the channel threshold back to the default value.

- When exporting Raw Channel Images or ROI Report, if status stalls at 99%, restart run.

- The **annotation in the image export may not reflect the correct settings** if they were previously altered (color mapping was changed or channel was hidden) in Render Settings.

- When **changing segment color**, if the color assignment in both the ROI tab and in the actual scan do not change as directed, refresh the page.

- The GeoMx **system may run out of memory** if attempting to **collect many ROIS on multiple slides**. Internally, this occurred during a run involving nine plate swaps. Restart Data Collection.

Completing the Run/nCounter Preparation

- **Check your plate after collection to ensure the expected wells were collected. The Plate Status Indicator may display an inaccurate status**. If the number or location of filled wells differs from your expectations based on the number of ROIs and segments collected, contact geomxsupport@nanostring.com.

- **After finalizing a row, its wells should appear as green** in the plate icon in the lower right of the GeoMx DSP Control Center. If not, refresh the page.

Data Analysis

- The system will not prevent more than one study from being created from a scan at the same time, however, an error will appear.

- Ensure your **scans have unique names,** particularly if you want to use the Manage Annotations function.

- If encountering the message, **"analysis is locked by another user"** check to see if another user is accessing this study. If not, close the study, wait one minute, refresh the page, then re-open the study.

- **P-value filter** may be ignored in trendline after toggling between "fold change" and "ratio" selections. **Reset filter** after changing selection.

- The **correlation calculation** will fail if a segment has the same count for all targets; this may occur if no targets have been detected and the count=1 for all. Remove these segments from the analysis.

- **Scan names should not have trailing or leading spaces**. This can cause segments from that scan to be unrecognizable in the manage annotations modal of Data Analysis. If spaces are accidentally introduced, revert to scan workspace, edit the scan name, then create a new Data Analysis study.

- In datasets and within Statistical Tests, when you click on **Trend Line**, all targets except the Negative are automatically **de-selected**. Navigate directly back to another plot (such as the volcano plot) and **manually re-select** all targets or targets of interest.

- **The GeoMx Auxiliary Server** needs to be **restarted** after a software update.
- If running the GeoMx Auxiliary Server and the GeoMx instrument is restarted for any reason, it will be necessary to restart the NanoString System Service on the auxiliary server, as well. This must be done after the GeoMx instrument has completed its start up.

- If you receive a **service unavailable** error when attempting to work with **the GeoMx Auxiliary Server**, first restart the GeoMx DSP instrument. Then, restart the auxiliary server via either the "NanoString System Service" or by restarting the server itself.

- The newly created segment group created in Filter: filter by segment may not appear in the segment group list until you refresh the page or close and reopen the study.

- In Manage Annotations, user-created column names should not start with "ROI", or they will be incorrectly associated with the ROI_ID and cause an error.

- In analysis with Illumina data, targets used for normalization will be included in statistical tests. If you would like them removed, you can manually de-select them before clicking Statistical analysis.

- When you expand the visualization pane in a cluster dendrogram, the software will not automatically expand into the space allowed. Zoom in until the plot fills the window, then zoom back out to the desired zoom level.

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GeoMx NGS Pipeline Software

Known Issues in GeoMx NGS Pipeline Software v1.0 or v2.0

- bcl to FASTQ file conversion software may be set to use custom headers; ensure the software you use to generate FASTQ files follows the FASTQ naming conventions set provided by Illumina, using the following link
https://support.illumina.com/help/BaseSpace_OLH_009008/Content/Source/Informatics/BS/NamingConvention_FASTQ-files-swBS.htm

- Some errors result in empty *.dcc* files. Check *.dcc* file sizes and the summary.txt file to ensure files were processed as expected. Rerun NGS Pipeline as needed to generate complete *.dcc* files.

- Ensure that your FASTQ files do not contain underscore characters (_) in the sequence identifier lines, as this may cause processing to fail, stall, or produce count files with errors. You may have underscores in your file names if your Illumina instrument name contains an underscore; if this is the case, modify your instrument name to remove any underscores. In addition, bcl to FASTQ file conversion software may be set to use custom headers; ensure the software you use to generate FASTQ files does not add any underscores to the sequence identifier lines.

- Do not modify FASTQ filenames from the Illumina FASTQ file naming conventions. If possible, retain the naming from the sample sheets exported from DSP, which incorporate the plate barcode. If you must change the sample names, ensure you do not add extra underscores, as the GeoMx NGS Pipeline software uses underscores to parse the Illumina-written portion of the file names to identify well/sample, lane, and read. In addition, if the sample name portions of your filenames are modified from the default pipeline workflow, you will need to update the names in your config file accordingly before you begin your NGS processing pipeline run.

- If inspecting your *.dcc* files in a text editor, you may notice lines for "tamperedIni,No" and "SOMEHASH100000000000". These are not currently functional and are placeholders for a future feature; they do not affect your data.

- Processing FASTQ files for only a subset of the ROIs listed in the GeoMx NGS Pipeline (DND) configuration file will result in empty *.dcc* files for the missing ROIs. Uploading these empty *.dcc* files will overwrite any data that may exist for these segments from a previous NGS Pipeline run. The GeoMx NGS Pipeline configuration file can be revised to exclude any ROIs for which FASTQ files were previously processed. The resulting *.dcc* zip uploaded should then only update segments associated with blank data.

Known Issues in GeoMx NGS Pipeline software v1.0 only (not applicable to v2.0)

- Submitting jobs from the GUI to a server may result in errors if there is already a run processing on the server.

- If logs from AWS batch run are not present, you may be able to view them on AWS cloudwatch. See documentation from AWS for more information.

- The GeoMx NGS Pipeline running on the server only listens on port 5000 and there is no argument to change this. This may cause a problem if there are other apps on server or network limiting the availability of that port.

- While the GeoMx NGS Pipeline is processing, it writes many large intermediate files to disc. Therefore, you will need up to 10x more space than your initial file set size on the computer where processing is occurring to complete processing. These intermediate files are removed automatically unless you modify the config file to "keepStepFile=True".

- When running on AWS batch, some jobs may silently fail, returning *.dcc* files with zero aligned and deduplicated reads or not returning some *.dcc* files at all, even though the AWS job console indicates that the job succeeded. Always review the summary.txt file to note any unexpected errors or zero reads for any intermediate steps for ROIs. Logs for a given job ID may be retrieved from AWS cloudwatch in order to troubleshoot.

- If executing a run from the GUI, any errors or failures will be indicated in the notification menu and listed by segment name.

- GeoMx Pipeline runs will fail if Docker Desktop is configured to use **WSL2**. To get around this issue, uncheck the *Use the WSL2 based engine* box on the **General** tab of the **Docker Desktop settings**.

- See below for a guideline for RAM requirements by sequencing data size to be processed.

Recommended compute resources for GeoMx NGS Pipeline

Recommended configurations (1 thread)

Read count	Configuration
0-10M	vCPU 2 RAM 4 GiB
10M-50M	vCPU 2 RAM 8 GiB
50M-100M	vCPU 4 RAM 16 GiB
100M+	vCPU 4 RAM 16 GiB

- Recommended settings are based on single thread run set up.
- Number of available threads depends on available cores.
- Required RAM will depend on number of threads and number of sequencing reads in the largest input file.

Resource usage (t2.xLarge)

Read count	Average RAM (GB)	Max RAM (GB)
0-10M (1 thread)	0.5	2.0
10M-50M	1.3	3.5
50M-100M	2.4	8.0
100M+	3.8	9.5

- Generally FASTQ file size is proportional to the total number of reads. However, the RAM requirement for a set of FASTQ files processed to a .DCC will be dependent on the target sequences with the most number of raw reads within the FASTQ files.

Recommended system settings based on sequencing data size

Read count \ Specification	vCPU 2 RAM 4 GiB	vCPU 2 RAM 8 GiB	vCPU 4 RAM 16 GiB		
0-10M	✓	✓	✓	Not recommended	x
10M-50M	○	✓	✓	Recommended	✓
50M-100M	x	○	✓	May have performance issues	○
100M+	x	x	✓		

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

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Figure 5: Recommended compute resources for GeoMx NGS Pipeline

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Idle State Pages

There are two idle states: **Collection Complete** & **Replace Plate**.

1. Click **Data Collection,** then **New/Continue Run**.

2. Follow on-screen instructions to load the instrument with a plate and empty slide holder.

3. Wait for pop-up message "No Slides Detected".

4. Click **OK**. Now, the instrument is at the Collection Complete idle state (Figure 6).
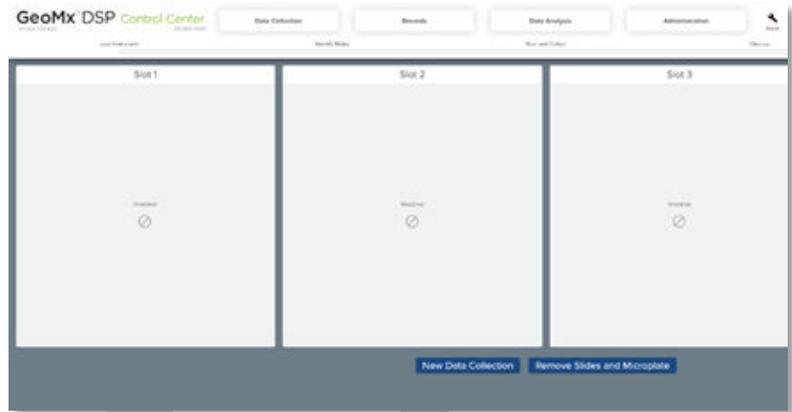

Figure 6: Collection Complete idle state

5. Click on reagent icons to change reagents or click on **Administration,** then **Software Update** to perform software update.

6. If you have followed these steps and the instrument still is not in an idle state, click **Assist** then **Restart Workflow**. Pause at "Replace Plate?" screen (Fig 2). This is an idle state.

7. If the instrument still does not allow these actions, power cycle by shutting down through the software, reboot, start a new run, and pause at "Replace Plate?" screen (Figure 7). This is an idle state.
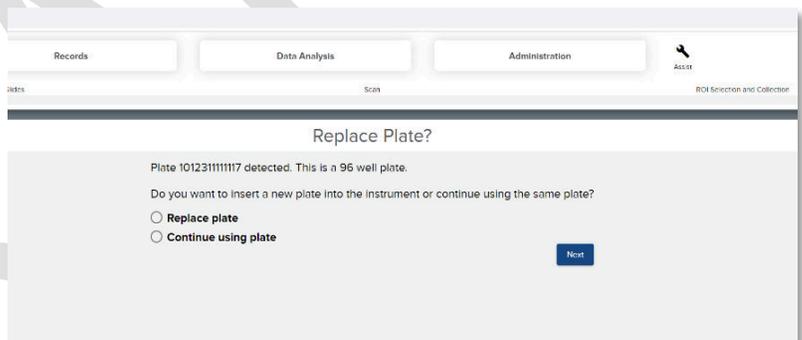

Figure 7: Replace Plate idle state