CosMx™ Spatial Molecular Imager

USER MANUAL

Instrument User Manual

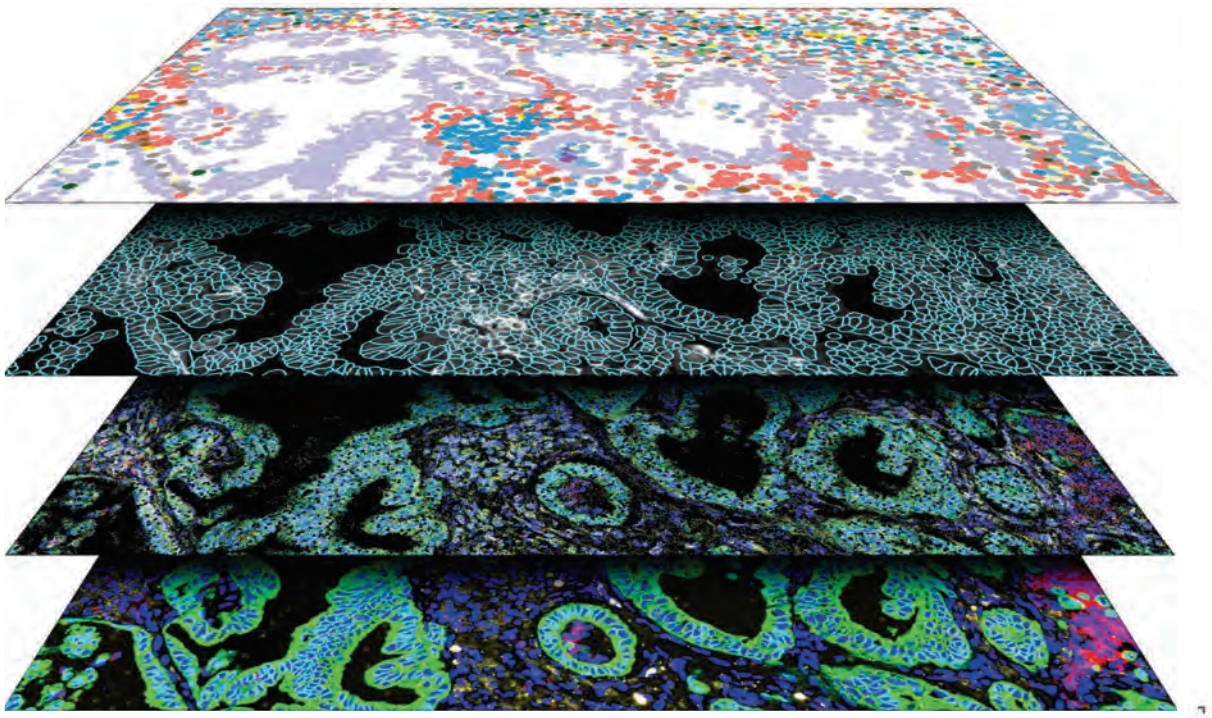

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Table of Contents

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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 and/or instructional material.

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

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.

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.

CosMx SMI User Manuals and Resources

The CosMx SMI workflow is divided into the following user manuals:

Workflow Step 1	CosMx SMI Manual Slide Preparation User Manual MAN-10159-01 CosMx SMI Semi-Automated Slide Preparation User Manual MAN-10160-01
Workflow Step 2	CosMx SMI Instrument User Manual MAN-10161-01
Workflow Step 3	CosMx SMI Data Analysis User Manual MAN-10162-01

User manuals and other documents can be found online in the NanoString University Document Library at https://university.nanostring.com.

Instrument and workflow training courses are also available in NanoString University.

For information about the AtoMx™ Spatial Informatics Platform, please refer to the AtoMx Spatial Informatics Platform User Manual (MAN-10170).

CosMx SMI Instrument Introduction

Figure 1: CosMx SMI Instrument

The CosMx Spatial Molecular Imager (SMI) **(see Figure 1)** is a benchtop instrument designed to provide multi-omics with any sample type at cellular and subcellular resolution. The CosMx SMI instrument is intended for Research Use Only. This **CosMx SMI Instrument Manual** is concerned exclusively with the operation of the CosMx Spatial Molecular Imager. It is important that you read and understand the provided information. Please use training resources in NanoString University at https://university.nanostring.com and contact Support@nanostring.com to address any questions or concerns.

Separate available user manuals provide information on slide preparation (manual and semi-automated) and data analysis. See **CosMx SMI User Manuals and Resources on page 7**.

Instrument Components

Figure 2: CosMx SMI instrument exterior diagram

The CosMx SMI instrument is a benchtop instrument and comes equipped with a 27" color UHD monitor (model: LG 27BN85U-B), as well as a mouse and keyboard.

The instrument upper door opens to the CosMx SMI flow cell nest, barcode scanner bay, and buffer bottles **(see Figure 3)**. The imaging tray bay door opens to load and unload the imaging tray or cleaning tray. The waste bay door provides access to the waste container.

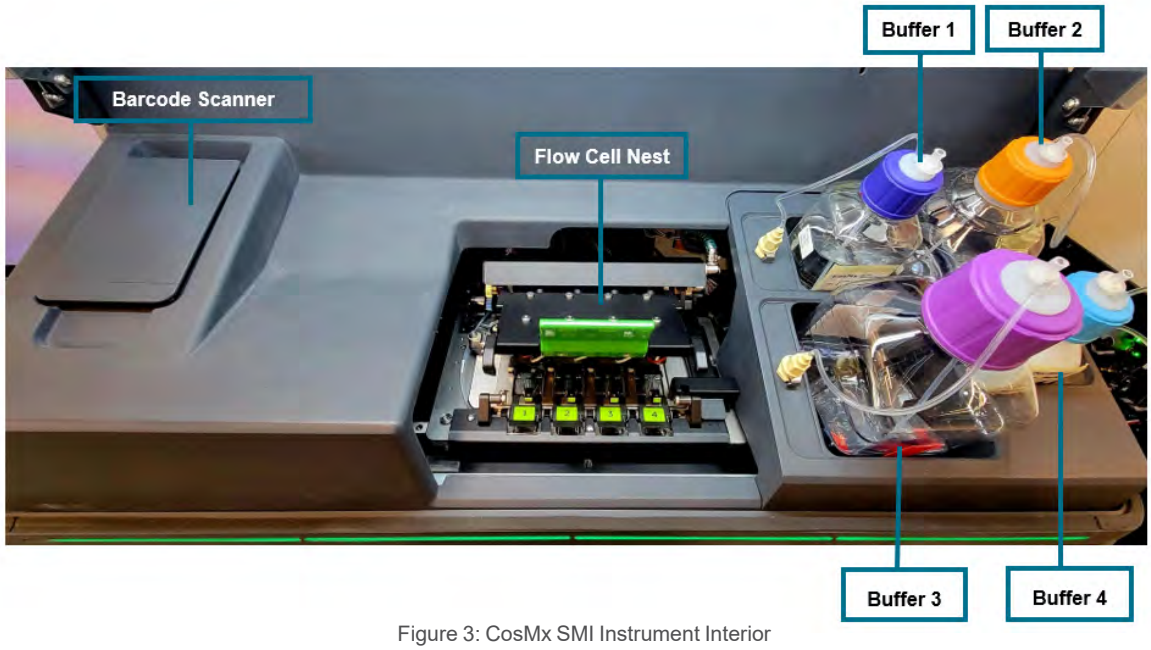


Figure 3: CosMx SMI Instrument Interior

To access the CosMx SMI Barcode Scanner Bay, Flow Cell Nest, and Buffers **(see Figure 3)**, open the Upper Door.

⚠ WARNING: Do not attempt to open the upper door while the instrument is running. The door will be locked unless the instrument is in idle state. Any attempt to bypass the main door lock will damage the instrument.

Up to four (4) flow cells can be loaded into the flow cell nest. The flow cells are secured into place by the flow cell nest clamp.

The CosMx SMI instrument requires the following single-use bulk reagent bottles to be loaded before starting a run **(see Figure 3)**. The waste bottle and colored reagent bottle caps are included with each instrument. **Do not dispose of the waste container or the provided colored caps.** All other consumables and reagents are **single-use and must be purchased separately**.

- Buffer 1: 500 mL bottle in reagent slot 1 (Purple)
- Buffer 2: 1 L bottle in reagent slot 2 (Orange)
- Buffer 3: 2 L bottle in reagent slot 3 (Pink)
- Buffer 4: 250 mL bottle in reagent slot 4 (Blue)

⚠ WARNING: Read the Safety Data Sheets (SDSs) and follow the handling instructions when handling bulk reagents and waste. Wear appropriate protective eye wear, clothing, and gloves. Safety Data Sheets (SDSs) are available from www.nanostring.com/support/support-documentation.

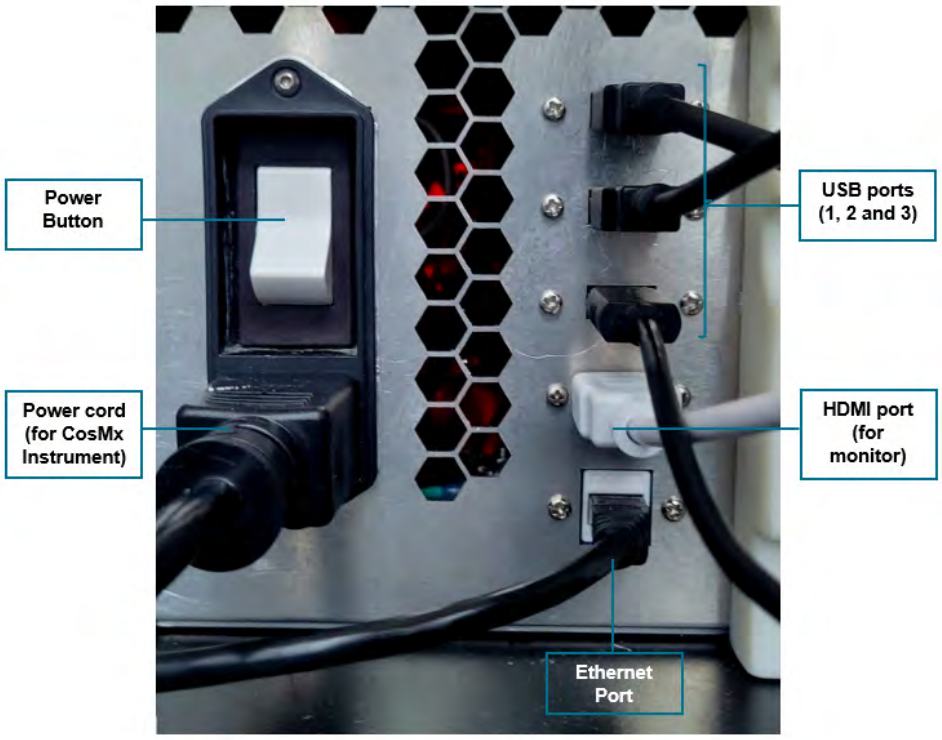


Figure 4: CosMx SMI instrument side panel diagram

The side panel of the CosMx SMI instrument **(see Figure 4)** has the following ports:

- An ethernet connection: Network Port with RJ45 connector. NanoString recommends using an ethernet cable with a rating of CAT6A or higher.
- Three USB connections: USB-A 3.0.
- A power input for the instrument: Power in 100 – 240 VAC, 50/60 Hz, connector C14.
- An HDMI port for the monitor.

Instrument Specifications

The CosMx SMI system is a stand-alone instrument, intended for Research Use Only. Instrument specifications are listed below **(see Table 1)**.

Table 1: CosMx instrument specifications

Slides per Run	Up to four (4) slides per run
Weight	280 lbs (127 kg)
Dimensions (W x D x H)	35 in x 29 in x 24 in (89 cm x 74 cm x 61 cm)
Power Requirements	880VA, 100-240 VAC, 50/60Hz
Room Temperature	18-28 °C
Room Humidity	10-85% relative humidity, non-condensing

Fluorescence Channel Specifications

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

Table 2: CosMx fluorescence channel specifications

Channel	Excitation (peak/wavelength)	Emission (peak/wavelength)
Blue	488 nm	512 nm
Green	530 nm	553 nm
Yellow	590 nm	630 nm
Red	656 nm	684 nm
UV	385 nm	-

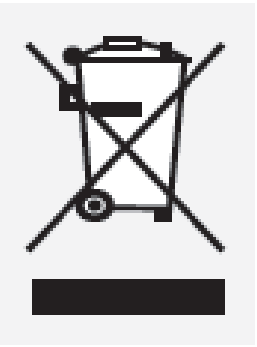
Installing and Moving

- Refer to the CosMx SMI Site Preparation Guide (MAN-10171) and the AtoMx Spatial Informatics Platform User Manual (MAN-10170) for detailed site and network requirements that must be met prior to the installation of the CosMx SMI instrument.
- Indoor use only. Avoid placing the instrument in a location with direct sunlight.
- The CosMx SMI instrument may only be installed or moved by appropriately trained personnel. Contact NanoString for installation or relocation of the instrument. See **Instrument Safety Information on page 15**.
- Place the instrument on a flat, stable surface with access to a dedicated power supply.
- Ensure adequate clearance on the backside of the instrument in order to access power controls and allow at least 3 inches for ventilation. **Unrestricted access to the instrument's rear panel is required for instrument service**.
- Ensure adequate clearance on the front of the instrument to allow for opening the waste and imaging tray compartments and to provide general lab access for movement of personnel around the lab.
- 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 CosMx SMI instrument that require additional instruction in this manual, however, some general safety guidelines are provided in **Instrument Safety Information on page 15** and in **Disposal of Electronic Equipment on page 14**.

Product Use Limitations

The CosMx SMI system is intended for research use only. Not for use in diagnostic procedures.

Disposal of Electronic Equipment



Review and follow all laws regarding the safe and proper disposal of electrical instrumentation. The symbol of a crossed out, wheeled bin on the product is required in accordance with the Waste Electrical and Electronic Equipment (WEEE Directive of the European Union). The presence of this marking on the product indicates that:

• The device was put on the European Market after August 13, 2005.

• The device is not to be disposed via the municipal waste collection system of any member state of the European Union.

For products under the requirement of the WEEE directive, please contact your 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.

Instrument Safety Information

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

If the CosMx SMI 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 CosMx SMI 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 or malfunction, and will void the manufacturer's warranty. Do not replace detachable main supply cords with 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 CosMx SMI instrument), use two or more people to lift the load.

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

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

WARNING: Connect the instrument power supply to a properly grounded receptacle with adequate voltage and current (see **Instrument Specifications on page 12**).

Caution Symbols

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

LASER WARNING: The CosMx Spatial Molecular Imager is a CLASS 1 LASER PRODUCT which includes lasers of a higher class whose beams are confined within a suitable enclosure so that access to laser radiation is physically prevented allowing safe

operation at all times when operated per the user manual. Operating the instrument with damaged or defeated door sensors, interlock circuits, or enclosure panels may expose the user to hazardous laser radiation.

Consignes de Sécurité (Français)

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

Ne pas tenter de démonter l'équipement. Le CosMx SMI 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 dedéplacer 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 CosMxSMI), 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).

Symboles d'avertissement

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 filtre du ventilateur.

AVERTISSEMENT LASER: L'imageur moléculaire spatial CosMx est un PRODUIT LASER DE CLASSE 1 qui comprend des lasers d'une classe supérieure dont les faisceaux sont confinés dans une enceinte appropriée afin que l'accès au rayonnement laser soit physiquement empêché, permettant un fonctionnement sûr à tout moment lorsqu'il est utilisé conformément au manuel d'utilisation. L'utilisation de l'instrument avec des capteurs de porte, des circuits de verrouillage ou des panneaux de boîtier endommagés ou défectueux peut exposer l'utilisateur à des rayonnements laser dangereux.

CosMx SMI Workflow Overview

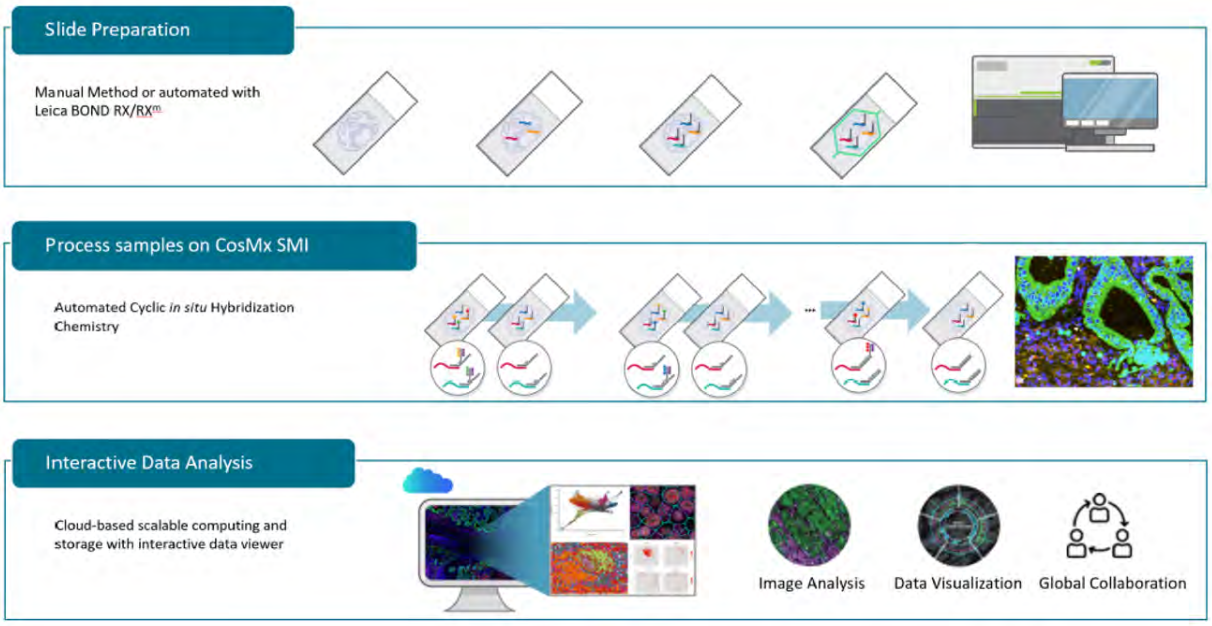


Figure 5: CosMx SMI Workflow Overview

Day 1: Slide Preparation. Prepare slides manually or using the BOND RX/RX^m fully automated IHC/ISH stainer from Leica Biosystems (BOND RX/RX^m).

Day 2: Process Slides on CosMx SMI. Complete assay and assemble the flow cells. Load assembled flow cells into the CosMx SMI instrument and enter flow cell/study information. Tissue is scanned to capture RNA or Protein readout and morphology imaging within user-designated fields of view (FOVs).

After run completion: Create a **Data Analysis** study in the AtoMx Spatial Informatics Platform (SIP) and perform quality-control checks, data analysis, and generate analysis plots.

CosMx SMI Control Center

The CosMx SMI Control Center is the main interface for the CosMx SMI instrument **(see Figure 6)**.

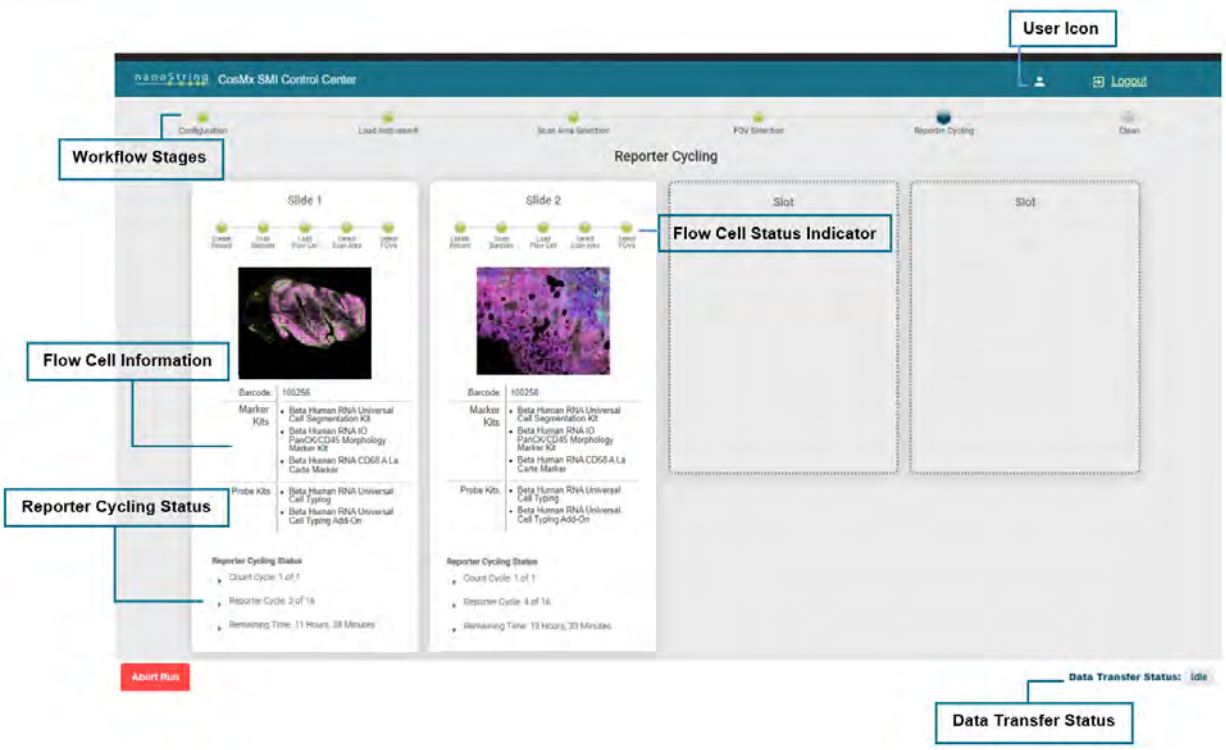


Figure 6: CosMx SMI Control Center

The **Workflow Stages**, shown just below the header, provides the user with a visual orientation of the total workflow progress.

The **Flow Cell Status Indicator,** at the top of the flow cell card, indicates the current status of each flow cell during run setup.

Also within the individual flow cell cards is the **Flow Cell Information**, which includes the Cell Segmentation and Marker Kit(s) and Probe Kit(s) selected during slide set-up.

Data Transfer Status shows the current data transfer status of the run data to AtoMx.

The **User Icon** displays the user name of the current signed in user.

See **Start a New Run (Acquisition) on page 24** for additional information on navigating through the CosMx SMI Control Center and setting up a CosMx SMI run.

CosMx SMI Run

Reagents and Consumables

IMPORTANT: Upon receipt of the NanoString Supplied Reagents and consumables, **open all packaging, verify reagents, and visually inspect the Imaging Tray and the Cleaning Tray for any damage** that may have occurred during shipping.

Once inspected, **return trays to their original packaging for storage**. Do not stack imaging or cleaning trays if not in original box as this could cause damage to the foil cover and may result in evaporation and cross contamination.

CosMx SMI Bulk Reagents Kit (Room Temperature)

Figure 7: CosMx SMI Bulk Reagents Kit

Table 3: CosMx Instrument Buffer Kit

CosMx RNA Buffer Kit, Small **2 slide**	**CosMx RNA Buffer Kit, Medium** **4 slide**	**CosMx Protein Buffer Kit**
CosMx Buffer 1	CosMx Buffer 1	CosMx Buffer 1
CosMx Buffer 2	CosMx Buffer 2	CosMx Buffer 2
CosMx Buffer 3, Small (550 mL)	CosMx Buffer 3, Medium (990 mL)	CosMx Buffer 3, Small (550 mL)
CosMx Buffer 4, Small (58 mL)	CosMx Buffer 4, Medium (90 mL)	CosMx Buffer 4, Small (58 mL)
Catalase, Small (4°C)	Catalase, Medium (4°C)	Catalase, Small (4°C)
Pyranose Oxidase, Small (4°C)	Pyranose Oxidase, Medium (4°C)	Pyranose Oxidase, Small (4°C)

CosMx Imaging Tray (4°C)

IMPORTANT: The CosMx SMI Imaging Tray is analyte, plexity, and slide dependent. Check the tray label before use to ensure the correct imaging tray has been selected.

Figure 8: CosMx SMI Imaging Tray

Table 4: CosMx Imaging Tray Options

RNA				Protein	
100-plex		1000-plex		64-plex	
2 slides	4 slides	2 slides	4 slides	2 slides	4 slides
CosMx RNA Imaging Tray (100 Plex, 2 Slide Run)	CosMx RNA Imaging Tray (100 Plex, 4 Slide Run)	CosMx RNA Imaging Tray (1000 Plex, 2 Slide Run)	CosMx RNA Imaging Tray (1000 Plex, 4 Slide Run)	CosMx Protein Imaging Tray (2 Slide Run)	CosMx Protein Imaging Tray (4 Slide Run)
CosMx RNase Inhibitor (2-Slide Run)	CosMx RNase Inhibitor (4-Slide Run)	CosMx RNase Inhibitor (2-Slide Run)	CosMx RNase Inhibitor (4-Slide Run)	CosMx RNase Inhibitor (2 Slide)	CosMx RNase Inhibitor (4 Slide)

CosMx Cleaning Tray (Room Temperature)

The included Cleaning Tray is blue in color and labeled as CosMx Cleaning Tray. Ensure the proper tray is used for the cleaning step.

Figure 9: CosMx Cleaning Tray

Start a New Run (Acquisition)

NanoString advises that users limit SMI Control Center access to only one (1) user at a time.

The ability to remote in to the instrument is limited by IT requirements and policies.

1. When first logging into the computer, enter the NanoString provided User Name and Password. Read through the NanoString Warning Statement **(see Figure 10)** and click **OK** to continue.

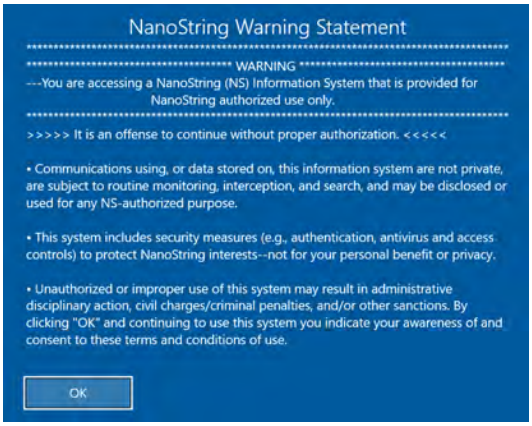


Figure 10: NanoString Warning Statement

2. If not automatically directed to the CosMx SMI Control Center login screen, select the **SMI Control Center** desktop icon on the desktop to open the SMI Control Center.
3. If prompted, **select Login** to continue to the Control Center Sign In page **(see Figure 11)**.

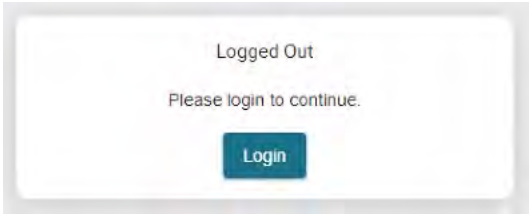


Figure 11: Select Login to Continue

4. Enter the Username and Password created during account set-up to sign in with Okta **(see Figure 12)**.

- The Username and Password are user specific and should have been set up during site preparation. For guidance on how to establish an Okta account with NanoString, see the AtoMx Spatial Informatics Platform Setup Guide.

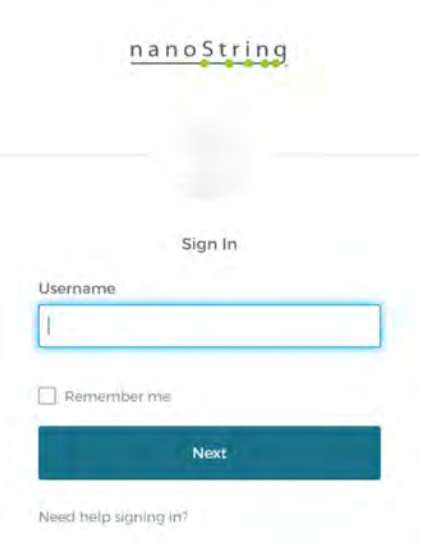


Figure 12: Sign in to Control Center

5. **Select Next** to continue to the CosMx SMI Control Center Home Screen **(see Figure 12)**.

CosMx SMI Control Center Home Screen

Figure 13: Control Center Home Screen

- **New Acquisition**: navigates to the flow cell configuration screen to begin a new run.
- **User Guide**: directs the user to NanoString University, which is a repository of User Manuals and CosMx SMI trainings that can be accessed by users after signing in with NanoString University credentials.
- **Admin Settings**: available for instrument administrative users. **Do not make any changes to the Admin Settings without direction from NanoString Support.**

Select New Acquisition to continue with run set-up.

Before beginning, remove the imaging tray from 4°C and leave at room temperature for a minimum of one (1) hour.

IMPORTANT: Do not remove the foil seal from the imaging tray. This could lead to cross-contamination between wells and affect experiment data.

For RNA runs, remove the RNase Inhibitor from -20°C and keep on ice until the step **Load the New Imaging Tray on page 42**.

Flow Cell Configuration

1. From the Flow Cell Configuration screen, **select Create Flow Cell (see Figure 14).**

Figure 14: Select Create Flow Cell

2. Create a **New Flow Cell Record** (fields marked with an asterisk (*) are required and cannot be left blank).

   Clicking outside of the Create Flow Cell window will close the window and any information that has been input will not be saved. Be sure to save all work before clicking outside of the window or closing the Control Center browser.

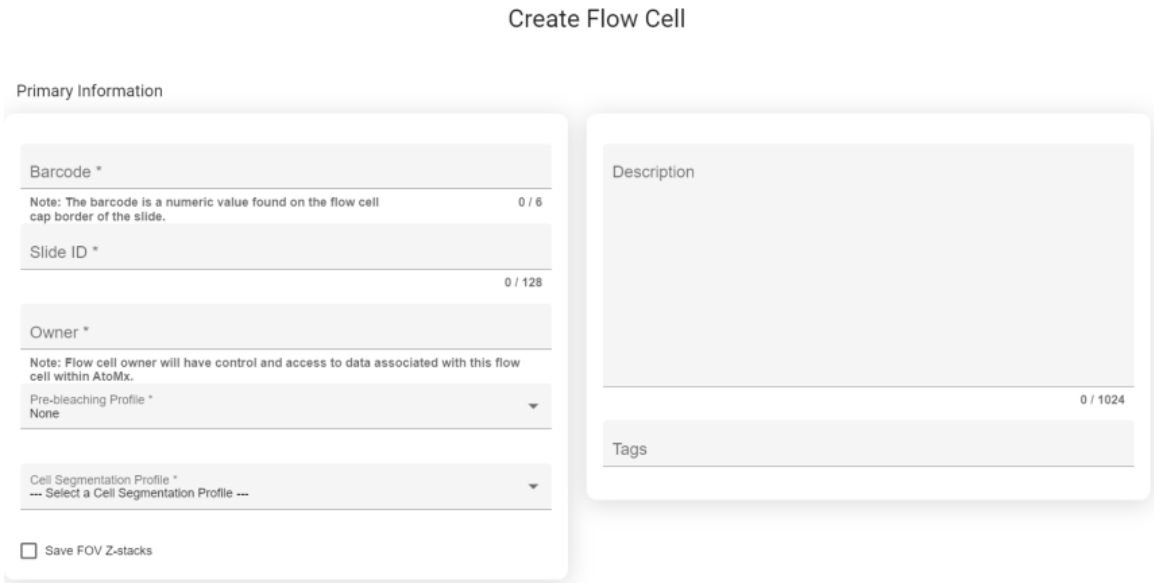


Figure 15: Create a New Flow Cell Record

- **Barcode**: the barcode is the six (6) digit numeric value found on the flow cell. The barcode is flow cell specific and cannot be used again once it has been scanned and loaded into the instrument.
- **Slide ID**: create a unique identifier for each flow cell.
- **Owner**: select the project / flow cell owner from the drop-down menu.
- **Pre-Bleaching Profile**: Use the table below **(see Table 5)** to select the appropriate Pre-bleaching Profile. Tissue type assumes FFPE tissue unless otherwise noted.

  Deviating from the recommended pre-bleaching profile will negatively impact data quality.

  If desired tissue type is not listed, contact Support@nanostring.com.

Table 5: Pre-bleaching Profile

Tissue Type	RNA	RNA	Protein
	Normal	Malignant	
Brain	Configuration B	Configuration A	Configuration C
Skin	Configuration A	Configuration A	Configuration C

Tissue Type	RNA	RNA	Protein
	Normal	Malignant	
Lung	Configuration A	Configuration C	Configuration C
Breast	Configuration A	Configuration A	Configuration C
Liver	Configuration B	Configuration A	Configuration C
Colorectal	Configuration A	Configuration A	Configuration C
Tonsil	Configuration A	Configuration A	Configuration C
Pancreas	Configuration C	Configuration C	Configuration C
Kidney	Configuration A	Configuration A	Configuration C
Fresh Frozen	Configuration C	Configuration C	Configuration C
CPA	n/a	n/a	n/a

- **Cell Segmentation Profile**: use the table below **(see Table 6)** to select the appropriate cell segmentation profile.

  Deviating from the recommended cell segmentation profile will negatively impact data quality.

  Some tissue types, including malignant tissue, may require additional optimization. Contact Support@nanostring.com for help optimizing cell segmentation.

Table 6: Cell Segmentation Profile

Configuration A	Configuration B	Configuration C	Configuaration D
Human Tissue	Neuro Tissue	Cell Pellet Array	Large Cell Types, typically associated with tumor cells.

- **Description**: the description section can be used as needed to include any project, flow cell and / or tissue specific information.
- **Tags**: tags can be used to organize flow cell record data in AtoMx Spatial Informatics Platform based on specific user-input key words. Flow cell tags could include labels such as "kidney", "Project 16", "training", etc.
- **Save FOV Z-stacks**: By default, CosMx SMI will generate Z-stacked images, or a composite image of all z-slices for a Field of View (FOV). If unstacked images are needed for additional post-run analysis (such as 3D segmentation, 3D visualization, and 3D analysis), check this box to save morphology images from all acquired Z-slices.

⚠ **WARNING**: Selecting to save FOV Z-stacks will result in additional cloud hosting charges and should only be selected if 3D imaging is required.

NOTE: If Save FOV Z-stacks is checked, a warning message will confirm to proceed with 3D Z Stack Image Archiving. **Press Proceed** to continue.

Figure 16: Select Proceed to confirm Z-stack Image Archiving

Segmentation and Reagent Configuration:

3. Using the drop down list, select the **Cell Segmentation Kit, Supplemental Marker Kit**, and **À La Carte Marker** (when applicable) used during slide preparation (MAN-10159-01 CosMx SMI Manual Slide Preparation User Manual) **(see Figure 17)**.

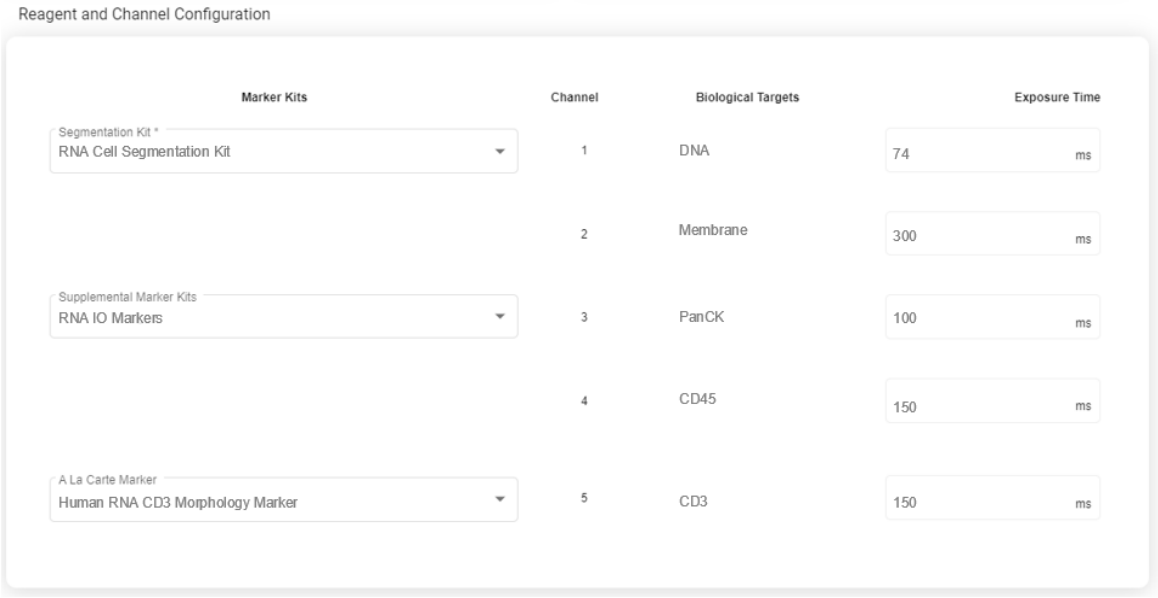


Figure 17: Marker Selection and Configuration

4. Once the Cell Segmentation and Supplemental Marker Kit selections are made, the **Channel, Biological Targets,** and **Exposure Time** fields will be automatically populated.

IMPORTANT: The exposure time fields are editable, however, **it is not recommended that these values be changed** unless advised to do so by NanoString. Changing these values manually will affect run performance and data acquisition.

5. Complete the **Reagent Configuration** field by selecting the Core Probe Panel and Add-ons (where applicable) **(see Figure 18)**.

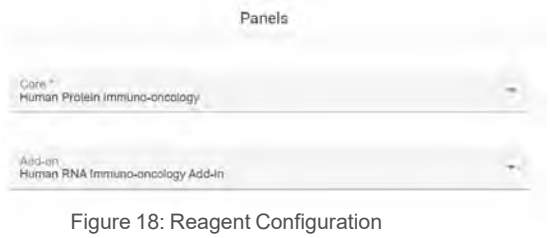


Figure 18: Reagent Configuration

IMPORTANT: Ensure that the correct panel configuration is selected for each slide as this will directly impact the genes analyzed from the assay. Add-on probes that come packaged with the core panel require a kit to be selected from the Add-on menu when used.

IMPORTANT: Flow cells within the same run must use the same reagent analyte (RNA or Protein). The analyte specific probe kit chosen to configure each flow cell must be compatible with the probe kits entered for every flow cell within the same run. The user will not be able to continue run set-up if conflicting analyte probe kits are selected.

6. Finally, fill in the **Tissue Information**. Fields marked with an asterisk (*) are required **(see Figure 19)**.
   - **Block ID**: Enter the block ID from the FFPE or Fresh Frozen block from which the tissue was sectioned.

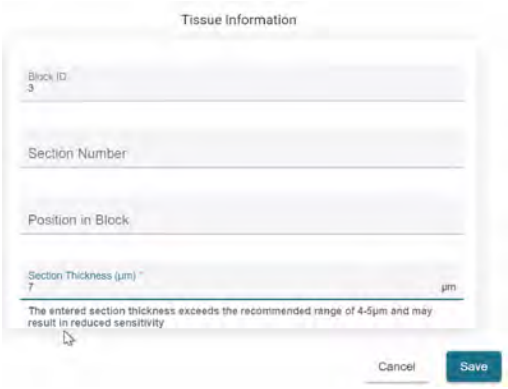


Figure 19: Tissue Information

   - **Section Number**: if known.
   - **Position in Block**: if known.
   - **Section Thickness (µm)**: enter the tissue section thickness. Five (5) µm is recommended for FFPE tissues. Fresh Frozen tissue should be 5-10 µm thick.

The tissue section thickness must be between 1 and 20 µm. Tissue sections outside of that range may cause fluidics errors during the run; therefore, if a value is entered that is outside of the range, it will not be accepted.

7. Once all fields have been completed, select **Save**.
8. Once the flow cell record has been saved, the user is directed back to the **Flow Cell Configuration Screen** **(see Figure 20)**.

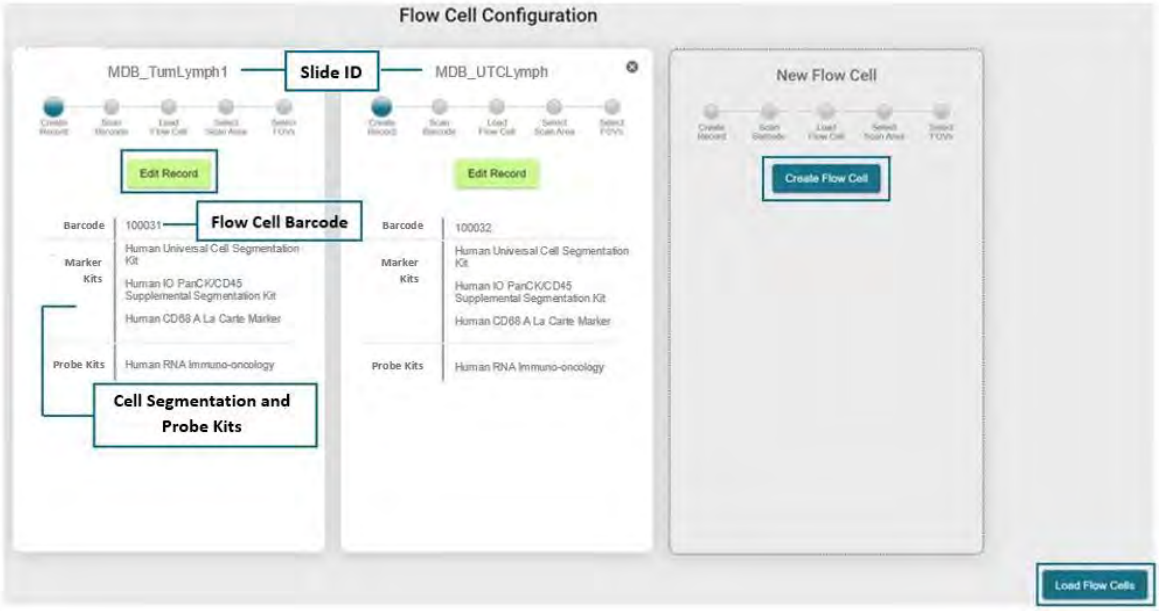


Figure 20: Flow Cell Configuration Screen

9. On the Flow Cell Configuration Screen, **ensure that all of the flow cell information is correct**. If changes need to be made, select the **Edit Record** button.
10. To add more flow cells to the run, select **Create Flow Cell** in the New Flow Cell card.
11. **Repeat steps 2 through 10 until all flow cells have been entered.**
12. Once all flow cells have been entered, select **Load Flow Cells** to continue.
13. A message will ask to confirm all flow cell information is complete. **Verify flow cell information and click Proceed to continue.**

Continue to **Load Instrument on page 34**.

Load Instrument

Empty Waste (if prompted)

If prompted, **empty and replace the waste container**.

1. **Release the fitting from the instrument** by pinching the gray button on the quick-release with thumb and forefinger (see Figure 21). The waste container cannot be removed from the bay until the quick- release fitting has been released.

Figure 21: Disconnect Waste Container

2. **Unscrew the cap** from the waste bottle and remove it.
3. **Dispose of the waste** according to laboratory waste disposal guidelines.

   WARNING: Instrument waste contains Proclin™ which is considered hazardous. Be sure to follow all waste disposal guidelines when emptying liquid waste.
4. **Screw the waste cap back on and load container back into waste bay.**
5. **Reattach the fitting**, continuing to press even after the first 'click' to ensure that the fitting is secure. The gray button will pop out when the bottle is secure.
6. Verify that tubing flowing into waste container has no kinks or obstructions and **close the waste bay door**.

Scan and Load the Flow Cells

Flow Cells should be fully assembled following the instructions found in the CosMx SMI Manual Slide Preparation User Manual (MAN-10159-01). Prior to loading flow cells, if an adhesive slide label was used, ensure the slide label is not folded over and is properly affixed to the slide.

Follow the CosMx SMI Control Center on screen prompts to scan and load the flow cells into the instrument **(see Figure 22)**.

1. Open the **Upper Door**.
2. Open the **Flow Cell Nest Lid**.

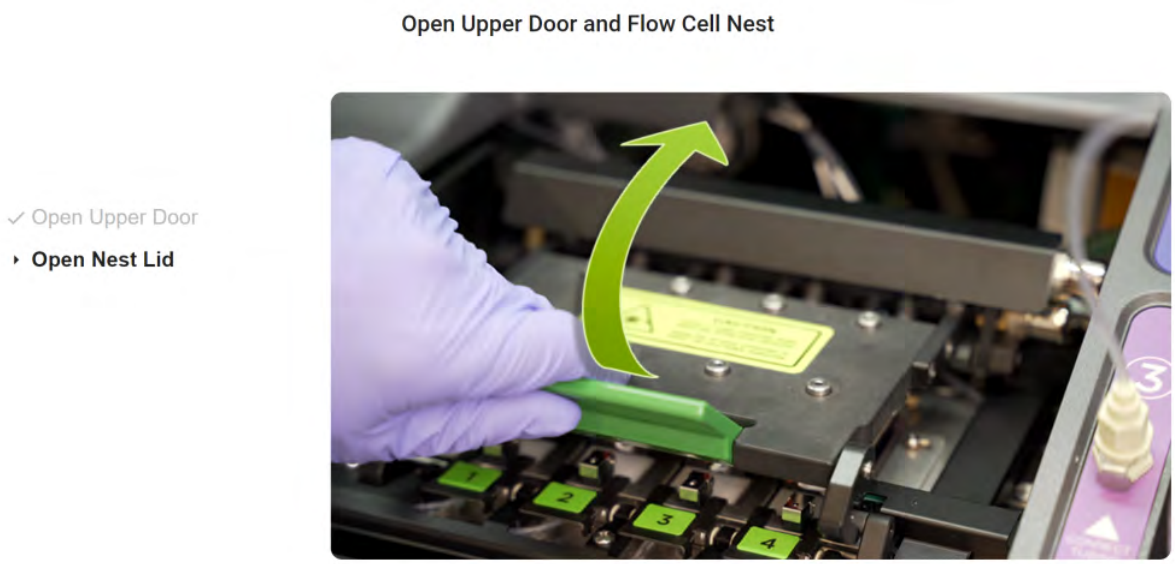


Figure 22: Open the Upper Door and Flow Cell Nest

3. Remove any used flow cells from the flow cell nest and dispose of, or store, according to laboratory guidelines.
4. When prompted, **open the barcode scanner bay door** on the left side of the instrument deck.
5. Place the flow cell **face down** into the barcode scanner bay.
6. The scanner will read the flow cell barcode and beep once the scanning is complete.

7. Move the flow cell from the barcode scanner and **place it face down, label side facing out, into any available slot** in the flow cell nest **(see Figure 23)**.

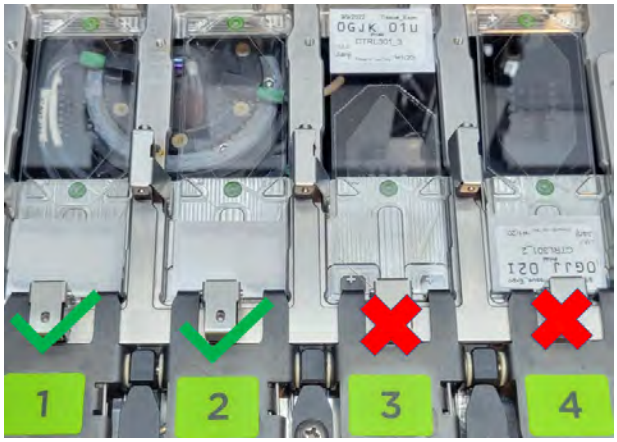


Figure 23: Load flow cells into nest. Flow cells in slot 1 and 2 are loaded correctly; flow cells in slot 3 and 4 are loaded incorrectly.

8. **Repeat** until all flow cells have been scanned and loaded.
   - Flow cells that still need to be scanned and loaded will show up in the top right of the screen in the **Remaining Flow Cells to Load** column **(see Figure 24)**.
   - As flow cells are scanned and loaded, the flow cell record information will be loaded into the appropriate slot.
   - Once all slides are loaded, **verify that all of the slide record information is correct** and that the slot location on the screen corresponds to the physical location of the flow cell in the flow cell nest.

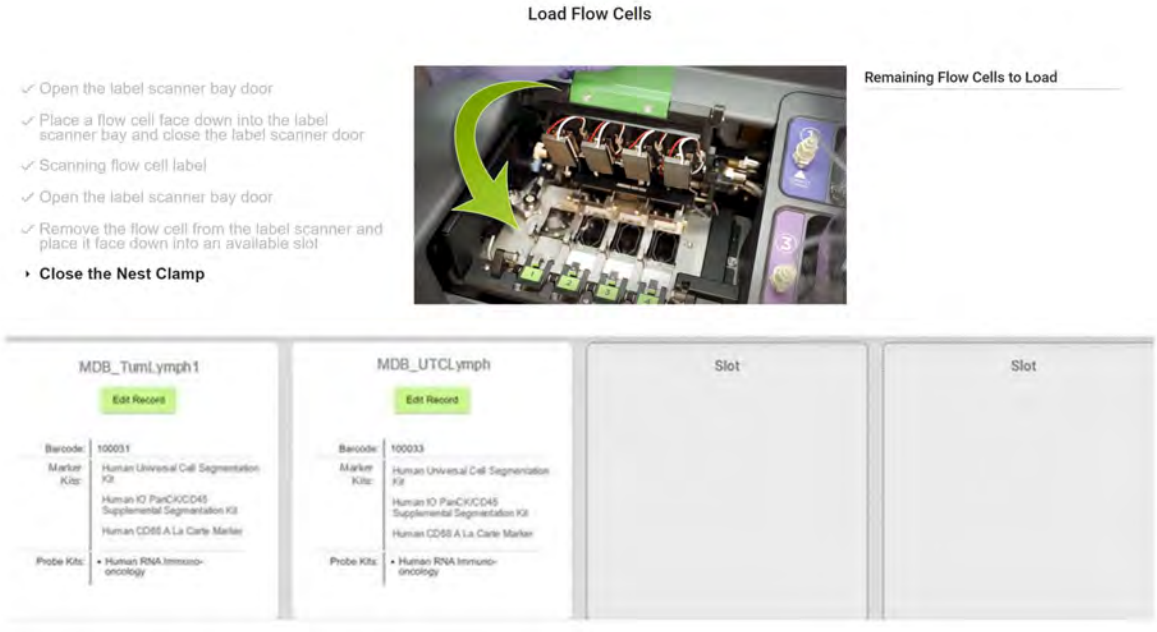


Figure 24: Load Flow Cells

IMPORTANT: Once the flow cells have been scanned and placed into the nest, do not remove the flow cells or transfer to a different slot. If a flow cell needs to be moved or removed, press *cancel* and follow the on-screen prompts to reload the flow cells.

9. **Close the flow cell nest lid and scanner bay door.**

Load Bulk Reagent Bottles

The CosMx SMI instrument requires **new bulk reagents** to be loaded into the instrument at the start of each run.

Remove lyophilized Catalase and Pyranose Oxidase (P20x) from 4°C and resuspend enzymes in DEPC-treated water:

- For a **2-slide configuration, add 250 µL of DEPC-water to each enzyme.** Vortex and centrifuge each tube**.** Add the entire volume (250 µL) into Buffer 4 before loading the buffer onto the instrument.
- For a **4-slide configuration, add 400 µL of DEPC-water to each enzyme.** Vortex and centrifuge each tube. Add the entire volume (400 µL) into Buffer 4 before loading the buffer onto the instrument.
- Mark Buffer 4 bottle after enzymes have been added.

Bulk reagent bottles are labeled 1-4 and color coordinated to indicate correct placement in the instrument (see **Reagents and Consumables on page 20**).

Follow the on screen prompts to remove used reagent bottles and load new bulk reagents. Bottles can be exchanged in any order.

1. **Remove the fitting from each bottle cap** by pinching the dark gray button on the quick- release with thumb and forefinger **(see Figure 25)**.

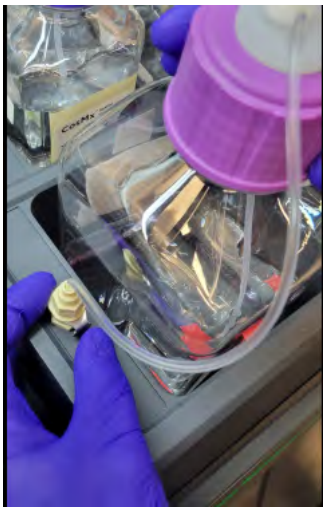


Figure 25: Disconnect Reagent Bottle

2. Remove all used bottles from the instrument and **transfer the colored cap from the used bottles to the new reagent bottles**. All four reagent bottles must be replaced to begin a new

run. Ensure the correct cap is used for each reagent.

IMPORTANT: Do not throw away the colored cap. This is considered part of the instrument and other caps cannot be used.

3. Place the new reagent bottle into the appropriate slot **(see Figure 26)**.

Figure 26: Load Bulk Reagent Bottles

4. **Reattach the cap fitting**, continuing to press even after the first 'click' to ensure that the fitting is secure. The gray button will pop out when the bottle is secure.
5. **Once all four (4) bottles have been changed**, all bottle status cards will show as **ready**.
6. Following the on-screen prompts, **close the nest lid** and **the barcode scanner door** (if not already closed), and **close the upper door.**
7. Once the door is closed, **confirm run parameters** for all flow cells and select **Validate Deck** **(see Figure 27)**.

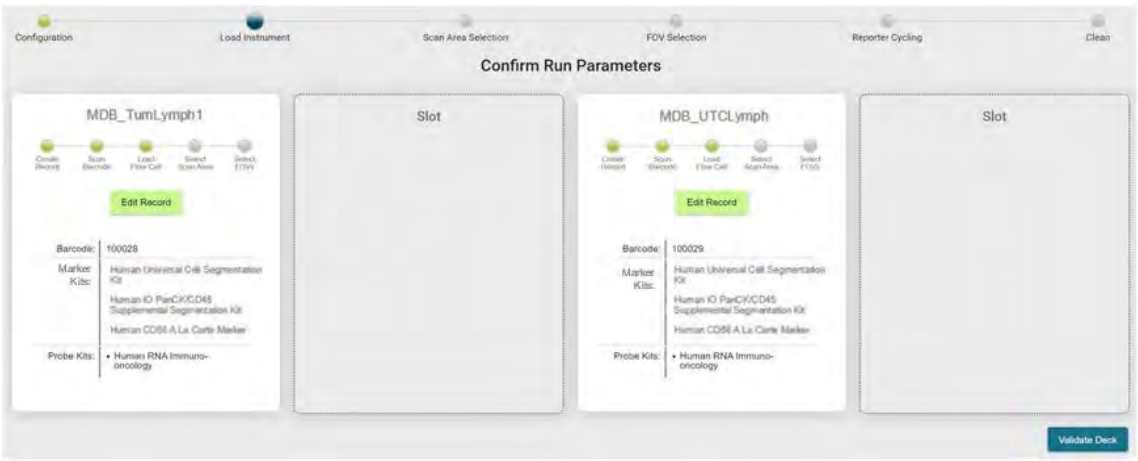


Figure 27: Confirm Run Parameters

8. Once Validate Deck is selected, a prompt will ask to "**Mark Flow Cells as Used?**" This is the final opportunity to review slide record and run information **(see Figure 28)**.

   - To go back and review or edit run information, select **Close**.

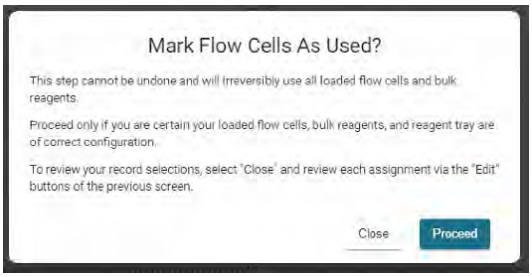


Figure 28: Mark Flow Cells as Used

   - To continue with the run, select **Proceed**. The run will only continue once Proceed has been selected.

After selecting to Proceed, the instrument will begin deck validation. The upper door will be locked and the instrument will check the connections and barcodes for the reagent bottles and verify the barcodes for each flow cell. Once the reagent and flow cell barcodes have been verified, they will be marked as used and cannot be used again for future runs. **Deck validation will take up to 30 minutes.**

IMPORTANT: After the flow cells have been marked as used, the *Cancel* button will change to *Abort*. Pressing *Abort* will exit the run set-up, mark all flow cells and reagents as used and either return the user to the Control Center Home Screen or prompt the user to begin a Post-Run Clean. The abort process may take up to 10 minutes.

If not already done, remove RNase Inhibitor from the -20°C freezer and leave on ice.

Dispose of used reagents following laboratory guidelines.

Load the New Imaging Tray

Before continuing, **ensure that the correct imaging tray is being used and that the imaging tray has acclimated to room temperture.** Using an incompatible tray will result in a critical error during loading.

- Verify analyte, plextity, and number of slides. See **Reagents and Consumables on page 20**.

IMPORTANT: Do not remove the foil seal from the reagent tray. This could lead to cross-contamination between wells and affect user data.

1. **If running a RNA Assay**, immediately before loading the imaging tray, **add RNase Inhibitor** to the well marked with the ⊕ symbol (well A11).
   - **Puncture the foil** of the marked well using a clean pipette tip.
   - **With a new pipette tip, add RNase Inhibitor** into the marked well.
     - For a **2-slide run, add 4 µL of RNase Inhibitor** into the marked well.
     - For a **4-slide run, add 8 µL of RNase Inhibitor** into the marked well.
2. Open the Imaging Tray Bay door and **remove the used tray**.
3. **Without removing the foil seal** from the imaging tray, load the new tray into the Imaging Bay ensuring the orientation is correct **(see Figure 29)**.

Figure 29: Load Imaging Tray

- Using the handle of the tray (the end of the tray marked with the ⊕ symbol), load the tray, label side first, into the instrument.
- Ensure that the imaging tray is properly loaded. When loading, slight resistance will be felt, continue pushing until tray is fully inserted. Tray is fully inserted when imaging tray legs line up with the internal grooves found in the imaging tray bay **(see Figure 30)**.

Figure 30: Ensure imaging tray is properly loaded

- Do not force the tray if it is not sliding into the imaging tray bay. Rather remove the tray and try inserting again until the tray slides into place.

4. Once the tray is loaded, **close the Imaging Tray Bay Door**.

Once the Imaging Tray Bay Door is closed, the instrument will validate hardware components and begin the tissue find scan. **This process may take up to 1 hour**.

Scan Flow Cells

Once the instrument has completed hardware validation, it will begin the **Tissue Find Scan**. Once the Tissue Find Scan has completed, the user will be directed to **select the scan area** **(see Figure 31)**. The defined scan area will be the basis for FOV selection. The total scan area per flow cell is 300 mm^2.

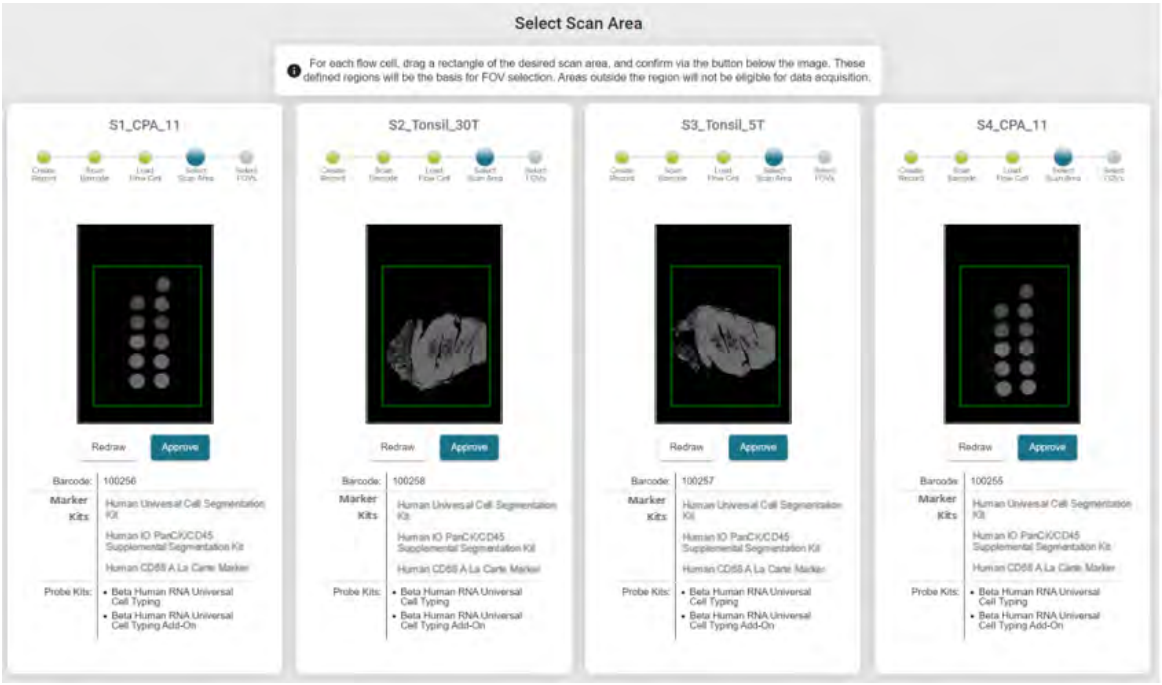


Figure 31: Select Scan Area

Define the Scan Area

The Tissue Find Scan result is displayed for each flow cell **(see Figure 31)**. The CosMx SMI Instrument will automatically detect tissue and set an optimal scan area, requiring only approval of each flow cell before proceeding. If needed, the scan area can be adjusted following the instructions below.

1. Using the computer mouse, click on the top-most corner of the desired scan area and **drag a rectangle around the tissue scan area**.

IMPORTANT: To reduce scan time, when defining the scan area, try to get as close to the tissue as possible without cutting off tissue. However, for small tissues, additional scan area may be needed to ensure the entire tissue area is captured.

2. Once tissue scan area has been selected, press **Approve**.
3. To change the tissue scan area, select **Re-draw** and draw a new rectangle around the desired area.
4. Once scan area is confirmed, select **Begin Scanning** to start the preview scan. The preview scan will take up to 1 hour.

Once **Begin Scanning** has been selected, the Begin Scanning button will grey out and a "please wait while scanning" message will appear. One the scan is complete, the instrument will advance to the FOV Selection screen.

Select Fields of View (FOVs)

The FOV Selection Workspace (see Figure 32) allows the user to position FOVs on the preview scan. Once FOVs have been selected, the CosMx SMI instrument begins Reporter Cycling and Data Acquisition.

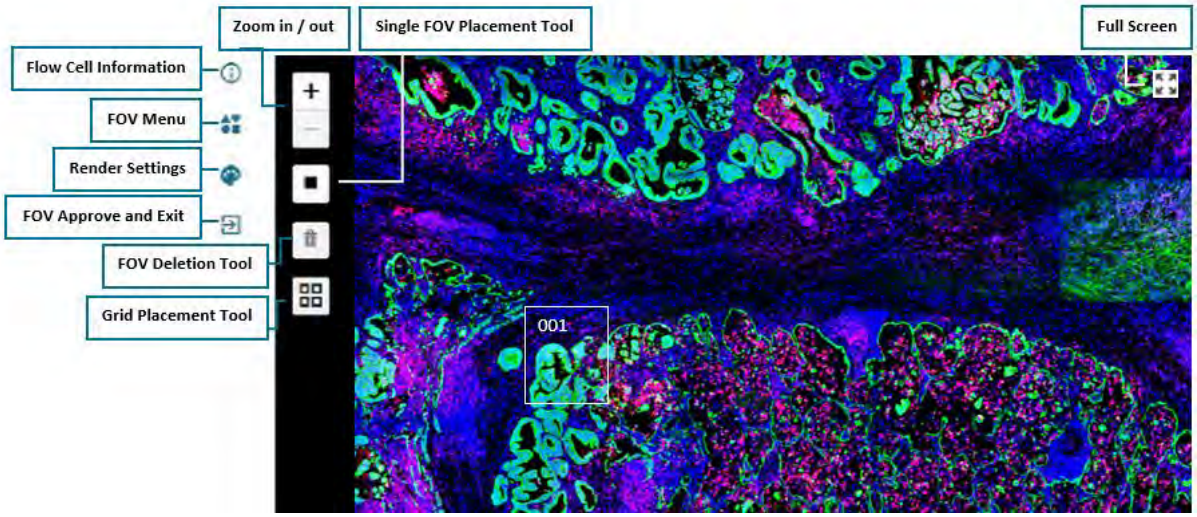


Figure 32: FOV Selection Workspace

Open the FOV Selection Workspace

From the FOV Scan Selection window, select **Edit FOVs** (see Figure 33).

Figure 33: FOV Selection Window

Inspect the Image

- Ensure that the scan quality is acceptable for designation of FOVs and segments. Do not proceed with collection from out of focus images, as it can cause poor or inaccurate results during data acquisition.
- Note that red blood cell autofluorescence is very common in FFPE tissues; be careful to differentiate nucleated cells from red blood cells (which do not contain nuclei).
- Open the **Flow Cell Information** 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 **Zoom In / Out** control. The scroll wheel on the computer mouse can also be used to zoom in and out.

- Open **Render Settings** (see Figure 34). 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.

  Note: the number of channels that appear in the Render Settings menu is dependent on the marker kit(s) selected during Flow Cell Configuration on page 27.

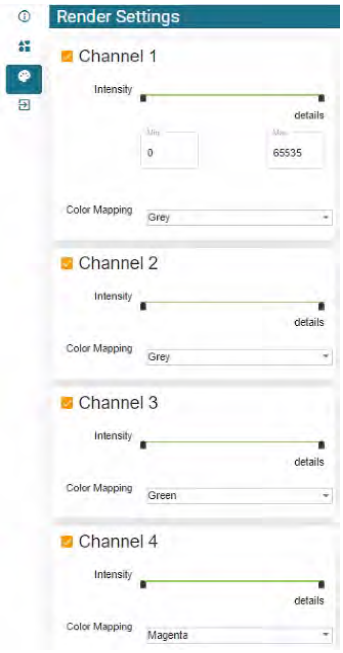


Figure 34: Render Settings Menu

Table 7: Channel Information

Channel Number	Channel Color
Channel 1	**Blue**
Channel 2	**Red**
Channel 3	**Green**
Channel 4	**Magenta**
Channel 5	**Cyan**

See **Instrument Specifications** for additional channel specifications.

Reference the **Slide Preparation User Manual** (manual or semi-automated) **CosMx SMI Cell Segmentation and Supplemental Marker Selection,** pages 12-13 for marker specific channel information.

Place FOVs

Choose from **single FOV placement** or **grid FOV placement** using the toolbar. The area for each FOV is 0.5 x 0.5 mm. Increasing the number of FOVs will increase instrument run time. NanoString recommends a run time of no more than 14 days and a total FOV area per experiment to be no more than 400 mm^2. For a 4-slide, 1000-plex RNA run, limit FOVs to 380 per flow cell (1,520 total FOVs) to keep the instrument run time within the 14 day recommendation.

IMPORTANT: Do not place FOVs in areas of the scan with poor focus or poor tissue quality as this can cause instrument damage and will affect data quality.

Single FOV Placement

1. Select the **Single FOV Placement Tool** from the FOV Selection Workspace (see Figure 35).

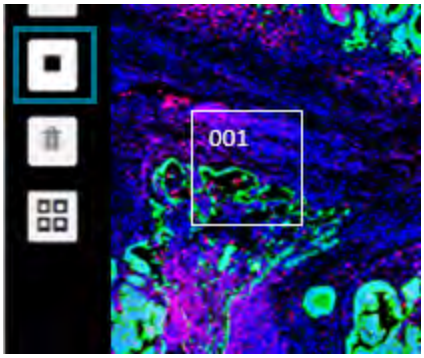


Figure 35: Single FOV Placement

2. Click in the **center of the area** that you would like to designate as a FOV.
3. FOVs can be moved as needed using the computer mouse to drag and drop.

   NOTE: only one (1) FOV can be moved at a time.

IMPORTANT: When placing FOVs, ensure that there is no overlap between FOVs as this could create run set-up errors and will affect data quality.

4. If a FOV needs to be removed, use the **FOV Deletion Tool** to delete the FOV.
   - Select the **FOV Deletion Tool** icon.

- Click on the FOV to be deleted. The box around the FOV will turn red.
- Once FOVs have been selected, click **Save**. The selected FOVs will be deleted.
- To delete all FOVs, select **delete all**.

5. Continue placing FOVs until all FOVs have been placed.
6. Once all FOVs have been placed, visually confirm placement location is correct before continuing.

Grid FOV Placement

1. Select the **Grid FOV Placement Tool** from the FOV Selection Workspace (see Figure 36).
   - The Grid FOV Placement Tool will open a window to allow the user to **set the number of FOVs per Column and per Row**.

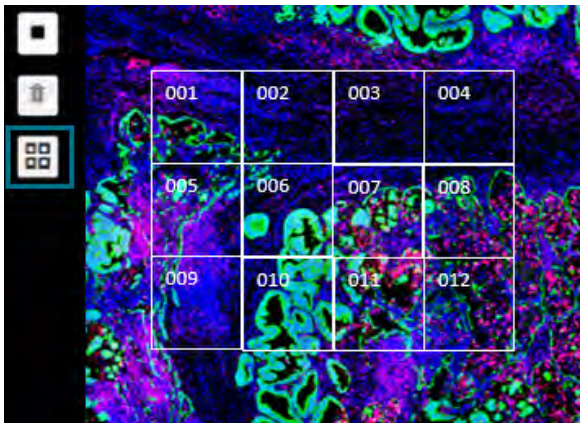


Figure 36: Grid FOV Placement

2. Click in the **center of the top left FOV of the grid** being placed (center of 001 (see Figure 36)).
3. FOVs can be moved as needed using the computer mouse to drag and drop.

   NOTE: only one (1) FOV can be moved at a time.
4. If a FOV needs to be removed, use the **FOV Deletion Tool** to delete the FOV.
   - Select the **FOV Deletion Tool** icon.
   - Click on the FOV to be deleted. The box around the FOV will turn red.
   - Once FOVs have been selected, click **Save**. The selected FOVs will be deleted.
   - To delete all FOVs, select **delete all**.

5. Once removed, continue placing FOVs until all FOVs have been placed.
6. Once all FOVs have been placed, visually confirm placement location is correct before continuing.

Approve FOVs

1. To review FOVs, select **FOV menu** (see Figure 37).
   - The FOV menu will show details on the X,Y axis location of the FOV for each Flow Cell.

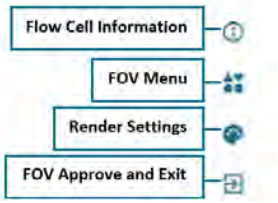


Figure 37: FOV Menu

2. After all FOVs have been placed and reviewed, select the **Approve and Exit** icon from the menu (see Figure 37).
3. Two options are available from the Approve and Exit selection:
   - **Save and Exit**: this option allows the user to save FOV placement but does not approve FOVs.
   - **Approve and Exit**: approving FOVs will change the FOV status to approved on the FOV selection menu. **All flow cells must have a status of FOV Selection Approval before reporter cycling can begin.**
4. Once all flow cells have been approved, the Begin Cycling button will turn blue and be active (see Figure 38).
5. **Click Begin Cycling** to start the run and begin reporter cycling.

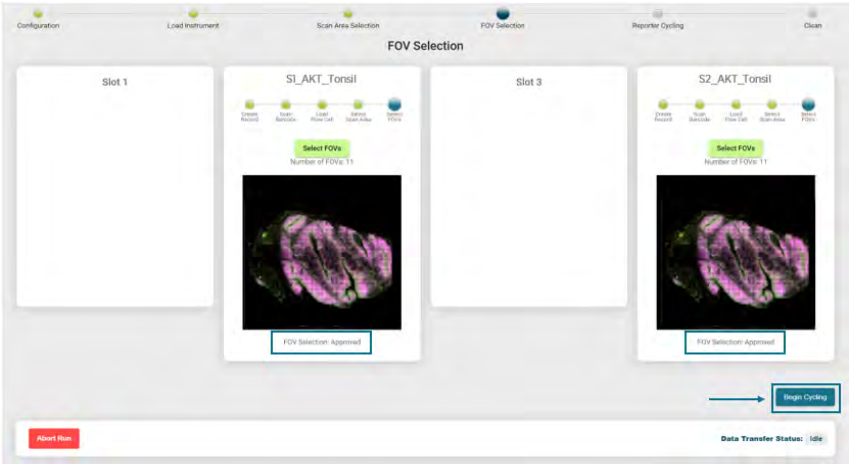


Figure 38: Begin Reporter Cycle

Reporter Cycling

Once **Begin Cycling** has been selected, reporter cycling will begin. The Reporter Cycling screen will now show **Reporter Cycling Status** and the Data Transfer Status will change to **In Progress**

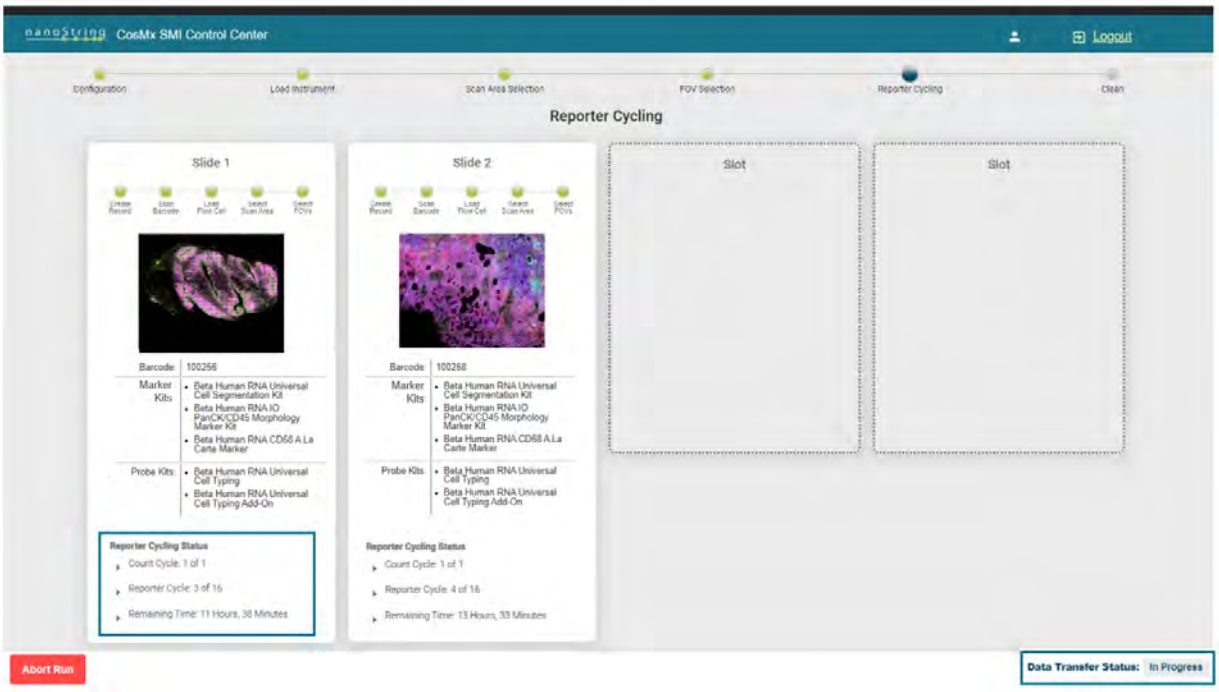


Figure 39: Reporter Cycling

The Reporter Cycling Status will show the **Count Cycle**, the **Reporter Cycle**, and the **Remaining Run Time.**

During the run, the run Transfer Status should say **In Progress**. However, if during the upload to AtoMx, one or more flow cells encounters an issue, the upload status indicator will change from **In progress** to **Error** (see Figure 40).

Figure 40: Data Transfer Error

To resolve the data transfer error:

- Expand the status indicator by clicking on the **expand arrows** to view the impacted flow cells.
- The data transfer status window will open and shows the status of each flow cell within the run (see Figure 41).
- To re-upload failed portions of the flow cell data to AtoMx, select **Retry** button.

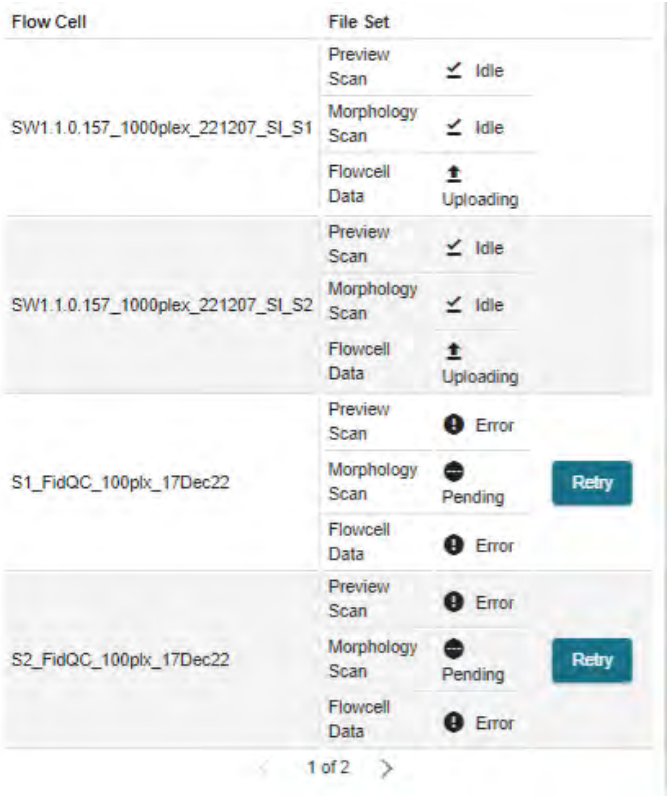


Figure 41: Data Transfer Status Window

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

Allow **30 seconds** between the time the button is pressed to the time the status updates from "Error" to "Uploading". If, after selecting Retry, the upload immediately fails again, wait **30 minutes** before reattempting. If the upload fails again, contact NanoString support at Support@nanostring.com.

NOTE: in cases of intermittent connectivity issues with AtoMx, this process could repeat multiple times during the course of a flow cell's upload. If you encounter what you believe to be an excessive number of returns to the error state, ensure networking conditions are stable and meet the IT requirements found in the Site Preparation Guide (MAN-10171), and that you are able to directly log into the AtoMx SIP. If you still encounter what you believe to be an excessive amount of error states during an upload, contact NanoString support at Support@nanostring.com.

If at any point during run set-up or reporter cycling the following error pop-up appears **(see Figure 42)**, select **Resume** to continue the run. If the issue continues, contact NanoString support at Support@nanostring.com.

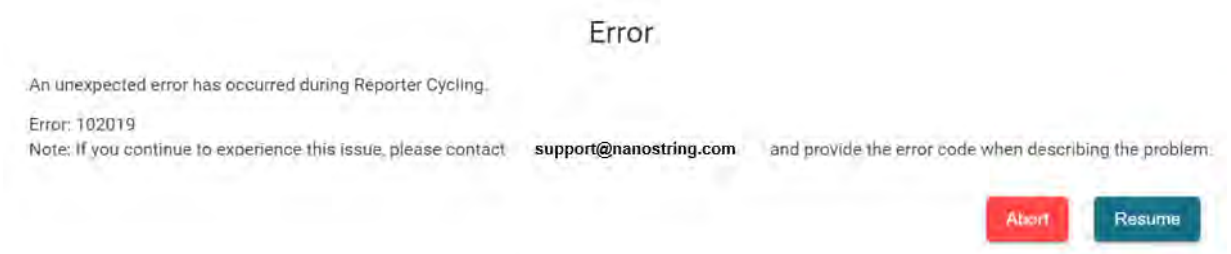


Figure 42: Reporter Cycling Error

Post Run Clean

Once reporter cycling has completed, the instrument will initiate a **post run clean**.

1. When instructed, open the imaging bay door and **remove the imaging tray**.
2. **Insert the blue pre-filled cleaning tray** and close the imaging bay door.
3. The instrument will run a cleaning cycle. **Do not attempt to open the instrument** while the cleaning cycle is running.

When the cleaning cycle has completed, follow the instrument prompts to **empty and replace the waste container**.

1. **Remove the fitting from the instrument** by pinching the gray button on the quick-release with thumb and forefinger (see Figure 43). The waste container cannot be removed from the bay until the quick- release fitting has been released.

Figure 43: Disconnect Waste Container

2. **Unscrew the cap** from the used bottle and remove it.
3. **Dispose of the waste** according to laboratory waste disposal guidelines.

   WARNING: Instrument waste contains Proclin™ which is considered hazardous. Be sure to follow all waste disposal guidelines when emptying liquid waste.
4. **Screw the waste cap back on and load container back into waste bay.**
5. **Reattach the fitting**, continuing to press even after the first 'click' to ensure that the fitting is secure. The gray button will pop out when the bottle is secure.

Follow on screen prompts to **unload the used flow cells**.

1. **Open** the upper door.
2. **Open** the flow cell nest lid.
3. **Remove** used flow cells and close the flow cell nest lid. **Do not remove bulk reagent bottles.**
4. If a more thorough cleaning is needed, continue to **Instrument Cleaning on page 56** for the instrument cleaning and decontamination procedure.
5. **Close** the upper door.
6. Dispose of or store flow cells according to laboratory glass disposal guidelines.

The instrument will return to the Control Center home screen.

Instrument Cleaning

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

Decontamination Guidelines

An EPA-registered disinfectant such as DNAZap™ from ThermoFisher Scientific shall be used to clean the equipment. If an off the shelf disinfectant is not available, freshly prepared 70% Ethanol or 70% Isopropanol can be used.

- An appropriate contact time of 70% Ethanol or Isopropanol solution is **20 minutes** in order to disinfect.

WARNING: Do not apply Bleach at any concentration to the instrument interior. Bleach will discolor the flow cell nest and may damage instrument components such as the barcode scanner.

1. Apply disinfectant to all exterior surfaces of equipment. If visible or gross contamination is present, apply enough disinfectant to saturate the contamination. Let stand for the manufacturers indicated contact time before wiping down.
2. Apply disinfectant to all reachable internal surfaces with a lint-free cloth keeping in mind to avoid any electrical components. Let stand for the indicated contact time before wiping down.

WARNING: do not apply excessive cleaning agents to instrument internals. Cleaning agents dripped into the instrument can cause damage.

WARNING: when cleaning the flow cell nest, use extra caution to not touch the objective or to allow disinfectant to drip onto the objective.

3. Repeat steps 1-2 with Deionized water to remove any residual disinfectant.
4. Discard contents of waste containers. Rinse container with 10% bleach solution and wait for 10 minutes then discard and rinse with Deionized water.
5. **Close** the upper door.
6. Dispose of or store flow cells according to laboratory glass disposal guidelines.

The instrument will return to the Control Center home screen.

Instrument Shut Down

Although not required, the CosMx SMI Instrument can be powered off using the power button on the side of the instrument. **Before powering off the instrument, ensure data acquisition and data transfer is complete**. The instrument control center should be on the home screen or flow cell configuration screen and the **Data Transfer Status must be Idle** (see Figure 44). Close the browser window and shut down the computer by navigating to the **Windows/Start** button on the PC, selecting **Power**, then **Shut Down**. Once the computer is shut down, the instrument can be powered off.

Figure 44: Data Transfer Status must be Idle

The cleaning tray and bulk reagents will remain in the instrument until the next run to prevent salt build-up in the fluidics lines and contamination.

Symbols and Definitions


Manufacturer

Authorized Representative in the European Community (Safety)


Safety CE Mark

Safety UK Conformity Assessed Mark

Catalogue or Reference Number

Batch code / Lot number

Serial number


Temperature range storage conditions


Lower limit of temperature storage conditions


Upper limit of temperature storage conditions


For Use by / Expiry Date


Date of Manufacture (DOM)

Regulatory Disclaimer

For applications for which approval/clearance is not required and research use.

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