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 may take **up to 1.25 hour**s.

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

Field of View (FOV) Selection Workspace

The FOV Selection Workspace (Figure 44) allows the user to position FOVs on the preview scan. Once FOVs have been selected and saved, the CosMx SMI instrument begins Reporter Cycling and Data Acquisition.

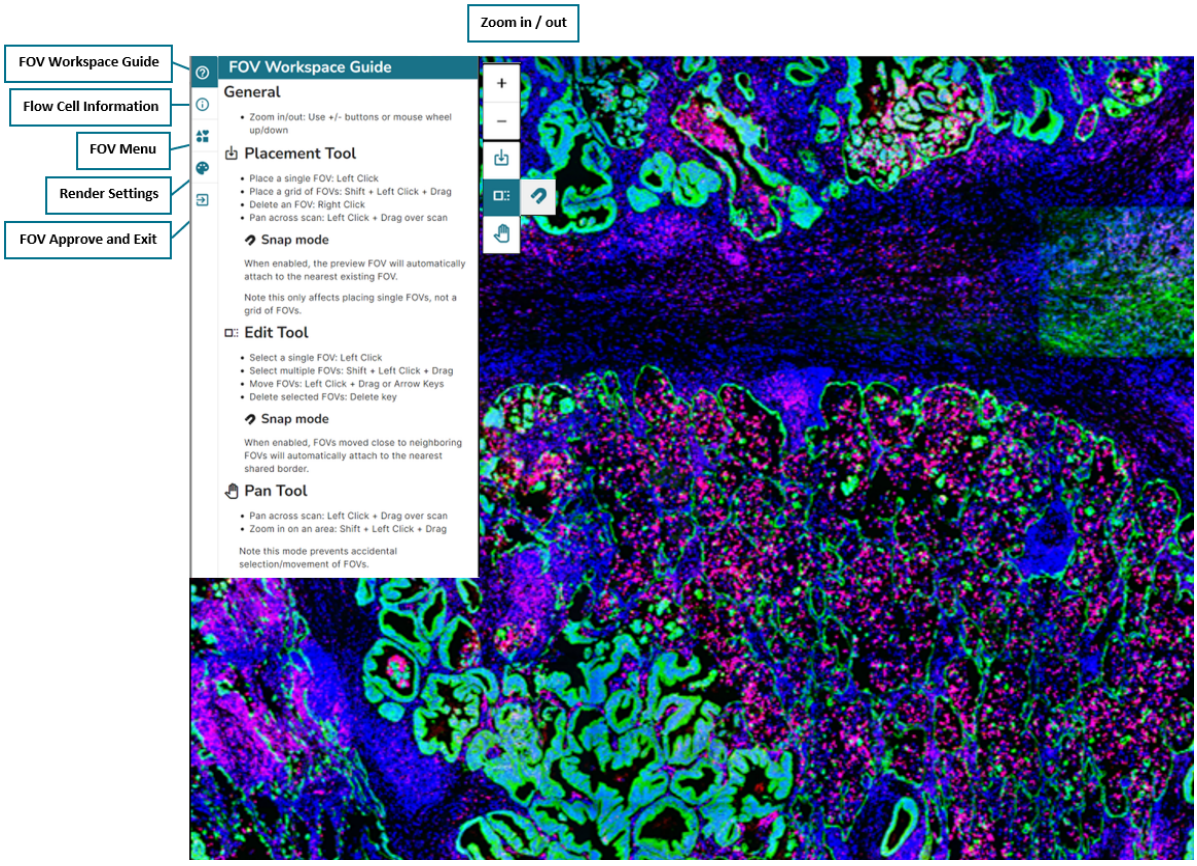


Figure 44: FOV Selection Workspace

Open the **FOV Selection Workspace:**

- From the FOV Scan Selection window, select **Edit FOVs** (Figure 45).

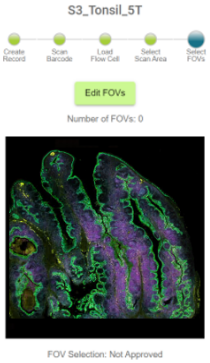


Figure 45: FOV Selection Window

Inspect the Image

- Use the image below to determine **tissue orientation** (Figure 46).

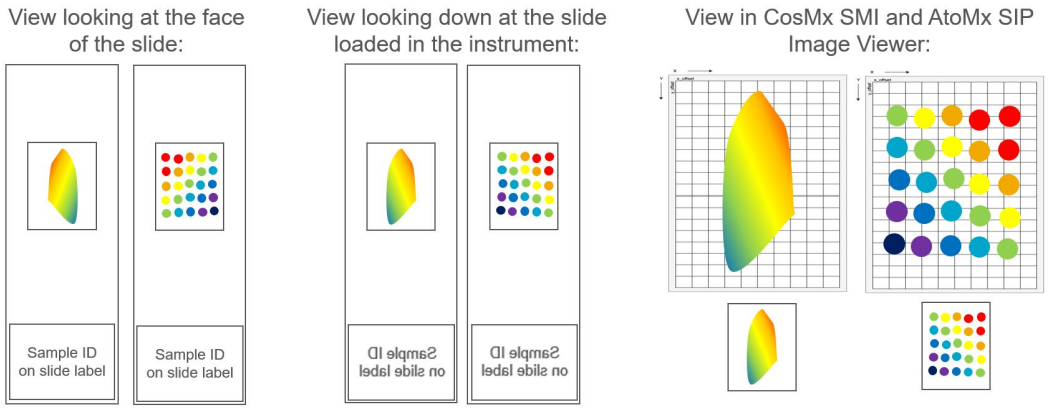


Figure 46: Tissue orientation

- Ensure that the scan quality is acceptable for the 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.
- 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** (Figure 47). 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.

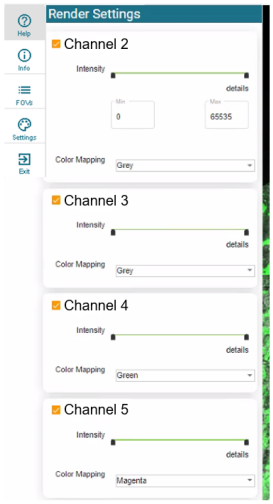


Figure 47: Render Settings Menu

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 37. Channel 1 is DAPI nuclear stain and will not be available during FOV selection.

Table 7: Channel Information

Channel Number	Channel Color	
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) **section on CosMx SMI Cell Segmentation and Supplemental Marker Selection,** for marker specific channel information.

Place FOVs

FOVs are placed in areas of interest using the **FOV Placement Tool**. The area for each FOV is 0.5 x 0.5 mm. Increasing the number of FOVs will increase instrument run time. For best performance, **NanoString recommends a run time of no more than 10 days**. Do not exceed a 14 day run-time as this may affect data quality. See the CosMx SMI Turnaround Time Estimation table on page 78.

> **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.

The **FOV Workspace Guide** provides detailed instructions for placing, editing, and deleting FOVs (Figure 44). To open the FOV Workspace Guide, select the Help (?) Icon.

FOV Placement Tool

Single FOV Placement

1. Select **Add** from the FOV Selection Workspace and left click in the **center of the area** to add a single FOV (Figure 48).

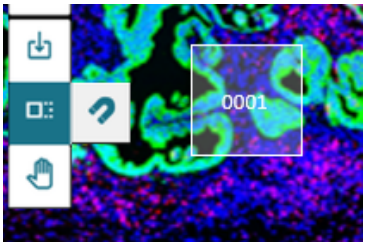


Figure 48: Single FOV Placement

2. Continue placing FOVs until all FOVs have been placed.

Snap Mode : when placing **single FOVs with snap mode enabled**, the new FOV will automatically attach to the nearest existing FOV (Figure 49).

Once the FOV is placed, it will snap into place and autonumber.

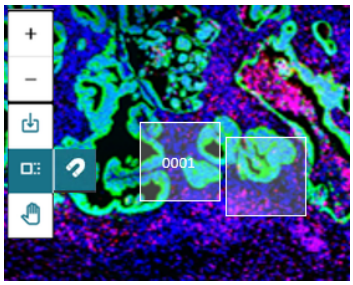


Figure 49: Snap mode

Grid FOV Placement

1. Select the **Add Icon** from the FOV Selection Workspace. Press **Shift** and left click on the **top left corner** of the desired grid location. Drag the mouse down and right to create the grid. A grid preview will display (Figure 50).

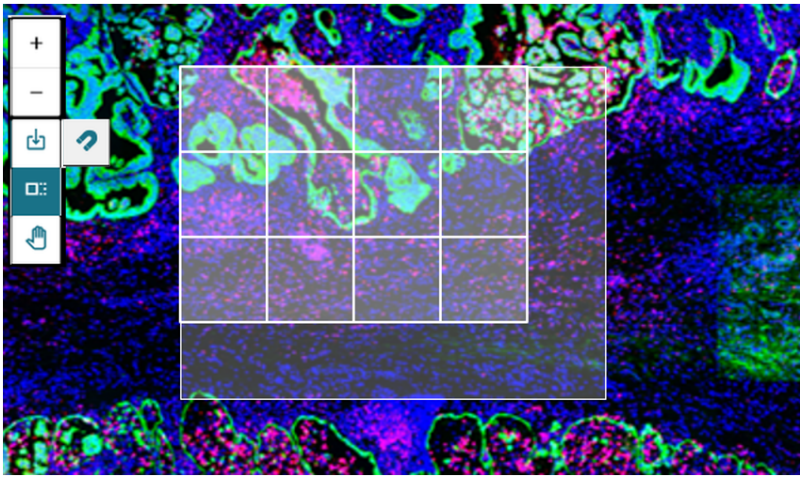


Figure 50: Grid Preview

2. Continue dragging until entire area of interest has the grid preview. Once desired area has been covered, **release the Shift key and the mouse to place the grid**. Once placed, FOVs within the grid will be autonumbered (Figure 51).

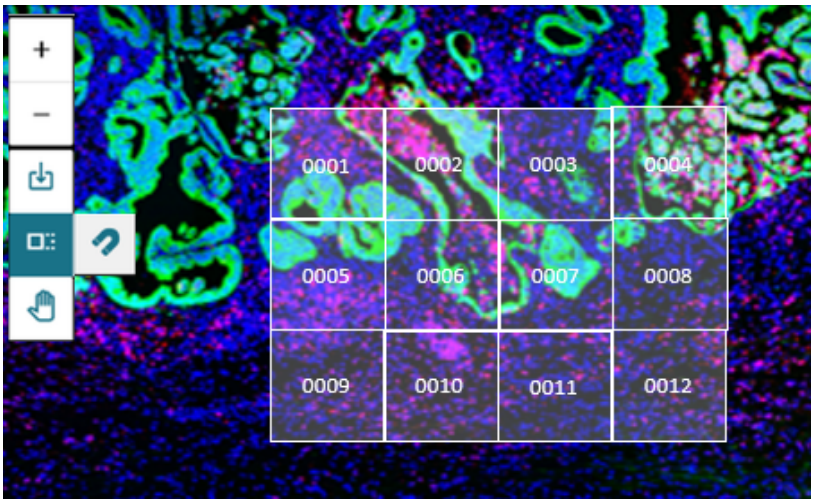
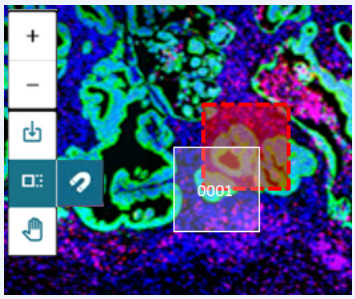


Figure 51: Grid FOV Placement

3. Continue placing FOVs until all FOVs have been placed.

> ⓘ**IMPORTANT**: when placing FOVs, the FOV outline will turn red if attempting to place an FOV in an invalid location, such as overlapping other FOVs or placement outside of the scan area. Move the mouse to a different location before placing the FOV. The FOVs cannot be approved if any invalid placements occur.
>
> 
>

Edit FOV Placement and Deleting FOVs with the Edit Tool

1. Select the **Edit** icon in the FOV Workspace.
2. **Select the FOV(s)** to be edited. Once selected, the FOV(s) will become grey with a yellow border.

   To select a **single FOV**, left click on the FOV to be edited.

   To select **multiple FOVs**:

   - ***Shift + Left Click + Drag*** will select multiple FOVs within a group by dragging across all of the FOVs to be edited.

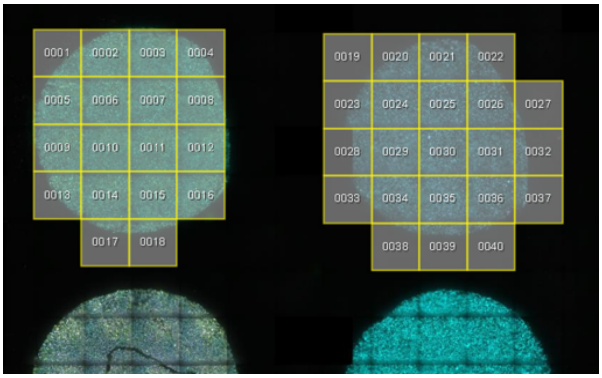


Figure 52: Select multiple FOVs by dragging to select

- ***Hold Ctrl + left click*** to select multiple FOVs not located next to each other.

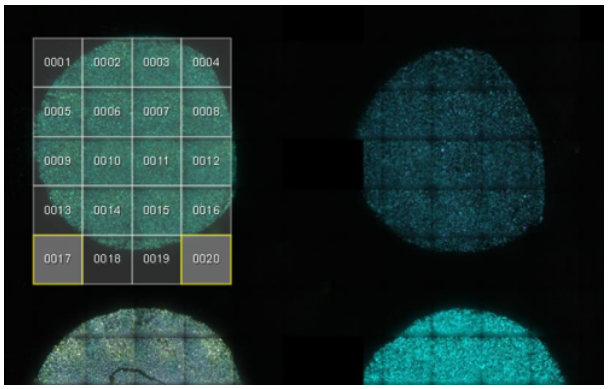


Figure 53: Select multiple FOVs one-at-a-time

3. To **delete** the selected FOVs, press the **Del** (delete) key on the keyboard. All selected FOVs will be deleted.
4. To **move** the selected FOVs, Left Click + Drag or use the keyboard arrow keys. All of the selected FOVs will move together.

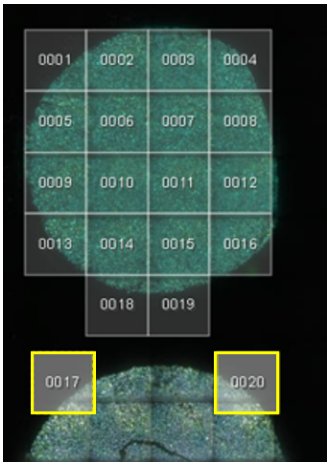


Figure 54: Selected FOVs will move together

Snap Mode can be enabled when moving FOVs by selecting the Snap icon ( ) beside the Edit icon. When Snap Mode is enabled, the FOV border of the FOV will be a dotted yellow line and FOVs moved close to neighboring FOVs will automatically attach to the nearest shared border.

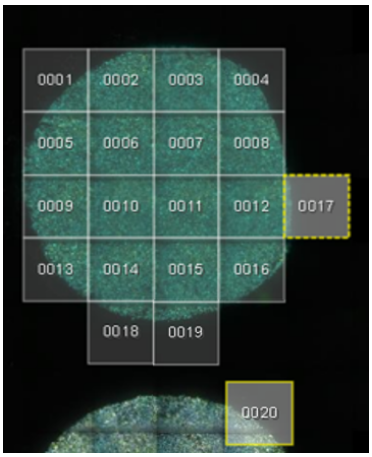
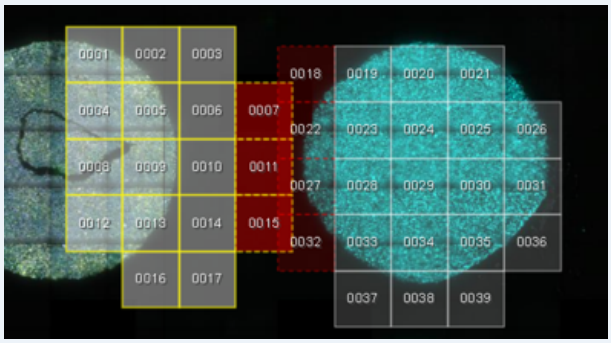


Figure 55: Snap Mode in Edit FOV

IMPORTANT: while in Edit mode, invalid FOV placement can be made. The invalid FOVs will show on the screen as red and have an error icon next to each affected FOV in the FOV Menu. These errors must be corrected before FOVs can be approved. This can be done by deleting or moving the FOVs within the Workspace.



Review and Approve FOVs

Review FOV placement prior to approving and exiting the FOV Workspace.

To review FOV placement on the tissue within the scannable area, the **Pan Tool** ( ) can be used.

- The Pan Tool allows the user to pan around the scan without risk of accidentally moving or deleting FOVs.

FOV Placement can also be reviewed in the **FOV List Pane**, accessed through the **FOV Menu** (Figure 56).

- The FOV list pane will show details on the **X,Y axis location** of the FOV for each flow cell as well as display a **reviewable error icon** next to invalidly placed FOVS.
    - To review the error, **hover over the error icon**.
- To highlight the invalid FOV(s) within the Workspace, **select the errored FOV (s)** within the FOV list pane. The selected FOV(s) will be highlighted in the Workspace and can be edited or deleted (see Edit FOV Placement and Deleting FOVs with the Edit Tool on page 63).

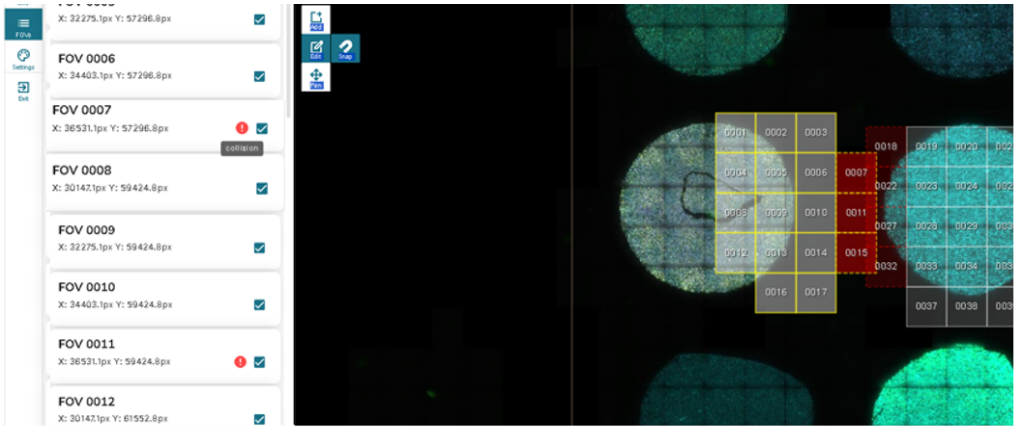


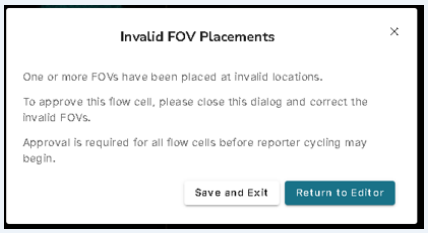
Figure 56: FOV List Pane with invalid FOV errors

Approve FOVs

1. After all FOVs have been placed and reviewed, select the **Exit** icon from the menu (Exit).

2. Two options are available from the **Exit** menu:

   - **Save and Exit**: this option allows the user to save FOV placement but does not approve FOVs for acquisition.
   - **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.**

   > ⓘ **IMPORTANT**: the system will warn the user if no FOVs were placed or if there are invalid FOVs (defined as 1 or more FOVs overlapping or outside of the scan area). Correct any errors before continuing. Once errors are corrected, continue to Approve and Exit.
   >
   > 
   >

3. Once all flow cells have been approved, the Begin Cycling button will turn blue and be active (Figure 57).
4. **Click Begin Cycling** to start the run and begin reporter cycling.