Region Selection Guidelines

During sample scanning, regions of interest must be defined prior to instrument run.

Key considerations with selecting regions

Refer to Region Selection for full list of guidelines

- The unit of selection is called a field of view (FOV). One FOV corresponds to one box in the grid.
- At least one FOV must be defined as a region. At least one region must be defined per slide. Regions do not need to be contiguous.



Each FOV can only be assigned to one region and cannot be selected twice. DO NOT select empty FOVs. Selecting empty FOVs will yield stitching errors.

- Avoid including FOVs that are <5% filled with tissue in a region. Inclusion of FOVs that are mostly blank can lead to stitching and registration errors
- Region names must be unique across all slides.
- For slides with multiple tissue sections:
 - Select each tissue section as a separate region
 - Exclude overlapping regions. If the overlapping region is assigned to one tissue, the overlapping area can be imaged but the data will be unusable.
 - No more than 8 regions can be selected per slide. If a slide contains more than 8 sections, consolidating some of these is recommended.



Region Selection

Initialize ~10-20 min
Load
~5 min
Load
1 h
Sample Scan
1 h
Region Sel.
~10 min
Cleanup
~5 min
Unload
~10 min
Cleanup
~5 min
Unload

a. Click "Add Region" to designate imaging area(s) for each slide cassette.



Each grid box is one field of view (FOV). FOV can only belong to one region selection and cannot be split or selected multiple times.



When selecting a region, **deselect all the blank FOVs**. Including blank FOVs will yield stitching errors.

b. Follow the guidelines for region selection. (Click "?" icon on top right bar to view key instructions)

Guideline	Example	
At least one region must be selected per slide. Region selection size: • Minimum: one FOV* • Maximum: entire well	Minimum size (one FOV)	Maximum size (entire well)
User defines number of tissue in each region. Sub-tissue section selection is not recommended		
Consolidating regions is recommended for slides with multiple sections Deselect blank FOVs. No more than 8 regions can be selected per slide	Correct	Incorrect
If unsure which tissue sections belong together, select all the sections as one region. <i>Deselect blank FOVs.</i>		
FOVs in a region do not need to be contiguous.		
Overlapping regions is not possible . If tissue sections overlap, exclude overlapping areas from either region.	Overlapping regions Do NOT select	Incorrect

Region names must be unique across all slides and contain only alphanumeric characters. Click pencil icon in the Regions window to edit. Region names are used to name the output directory, in the analysis summary HTML, the metrics_summary. CSV, and the experiment.xenium.