

caMicroscope Process Guide

ROI Selection

1. Double-click a location to create or reposition an ROI.
2. Select **10** ROIs per WSI. Target diverse morphology and sTILs density (especially high sTILs density) while distributing ROIs across entire tissue. Each ROI can satisfy multiple targets. Not all targets can be satisfied in each WSI. The numbers to select mentioned below are guides. In each WSI:
 - a. Select 3 ROIs inside tumor with stroma
(1 ROI should be at least 25% void of tissue)
 - b. Select 2 ROIs at invasive margin if discernable with stroma
(not sure this is possible with biopsies, 1 ROI should be at least 25% void of tissue)
 - c. Select 2 ROIs inside tumor or at margin **without stroma**
(not sure there are many or any of these)
 - d. Select 2 ROIs where there is no proximal tumor
(normal tissue: outside 500 um tumor margin)
 - e. Select 2 ROIs for each for the 16 pitfalls listed. The pitfalls were identified during sessions to understand pathologist variability and improve pathologist training and instructions for the assessment of sTILs [cite: Garcia2022_Cancers_v14p2467]. The pitfalls are discussed and exemplified in the [CME course](#), the test with feedback, and the [reference document](#).

Pitfalls:

benign glandular elements	cross-sectionally cut fibroblasts
adipocytes	low grade and/or degenerate ischemic tumor cells
carcinoma in situ	crushed cells
necrosis and fibrin	sparsely distributed tumor cells
nerves and/or larger caliber blood vessels	fibers
eosinophilia	folds
small/pyknotic nuclei	over-staining
perinuclear clearing	under-staining

3. Provide Annotations on selected ROIs.
 - a. HTT sTILs Study Annotations:
 - i. Evaluable versus Not Evaluable
 - ii. % Tumor-Associated Stroma
 - iii. sTILs Density
 - b. Tissue Type:
 - i. "Tumor with Stroma" and "Invasive Margin" are options when the ROI Type is "Evaluable".
 - ii. "Inside tumor or margin with low or no stroma" and "No Proximal Tumor" are options when the ROI type is "Not Evaluable for sTILs".
 - c. Pitfalls present: click the corresponding checkbox.