Recommendations for assessing tumor-infiltrating lymphocytes (TILs) in solid tumors in the residual disease setting

Guidelines for TILs assessment from the «International Immuno-Oncology Biomarker Working Group»

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1. Identify the area for TIL evaluation

- Assessment to be performed within the borders of the residual tumor bed.
- The area of the residual tumor bed is defined as the largest cross-sectional area between residual invasive cells (consistent with the definition used for calculating the Residual Cancer Burden RCB).
- The entire largest cross-sectional area of the residual tumor bed should be used for histologic TIL assessment.
- In case of pCR, TILs may, for specific research purposes, be assessed in the area of regression as defined by imaging, macroscopic and microscopic findings.

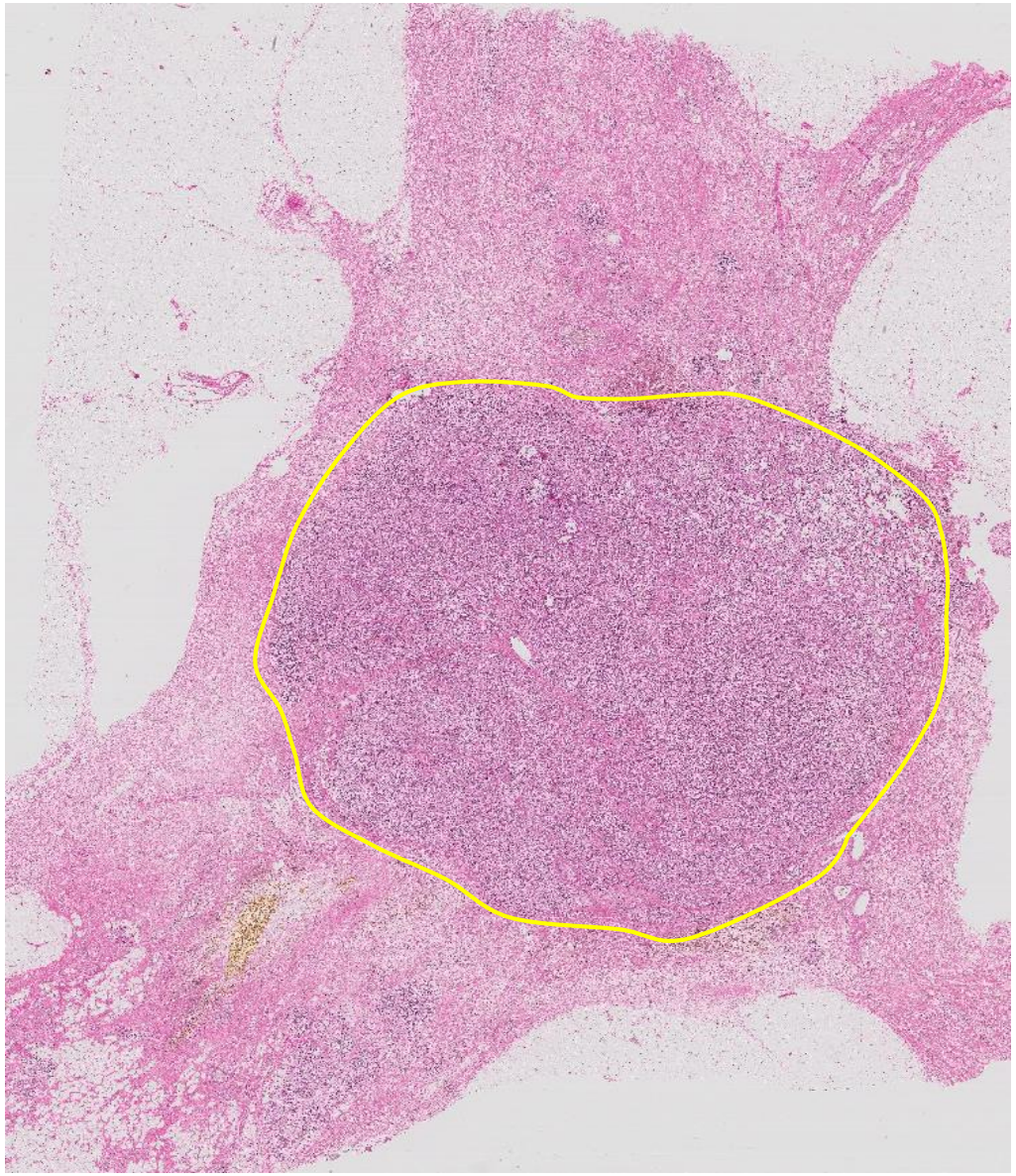
1. Identify the area for TIL evaluation

- One section (4-5 µm) per patient can be considered to be sufficient for practical purposes.

- If the residual tumor bed in case of non-pCR and the area of regression in pCR is only 2 cm one slide is considered enough.

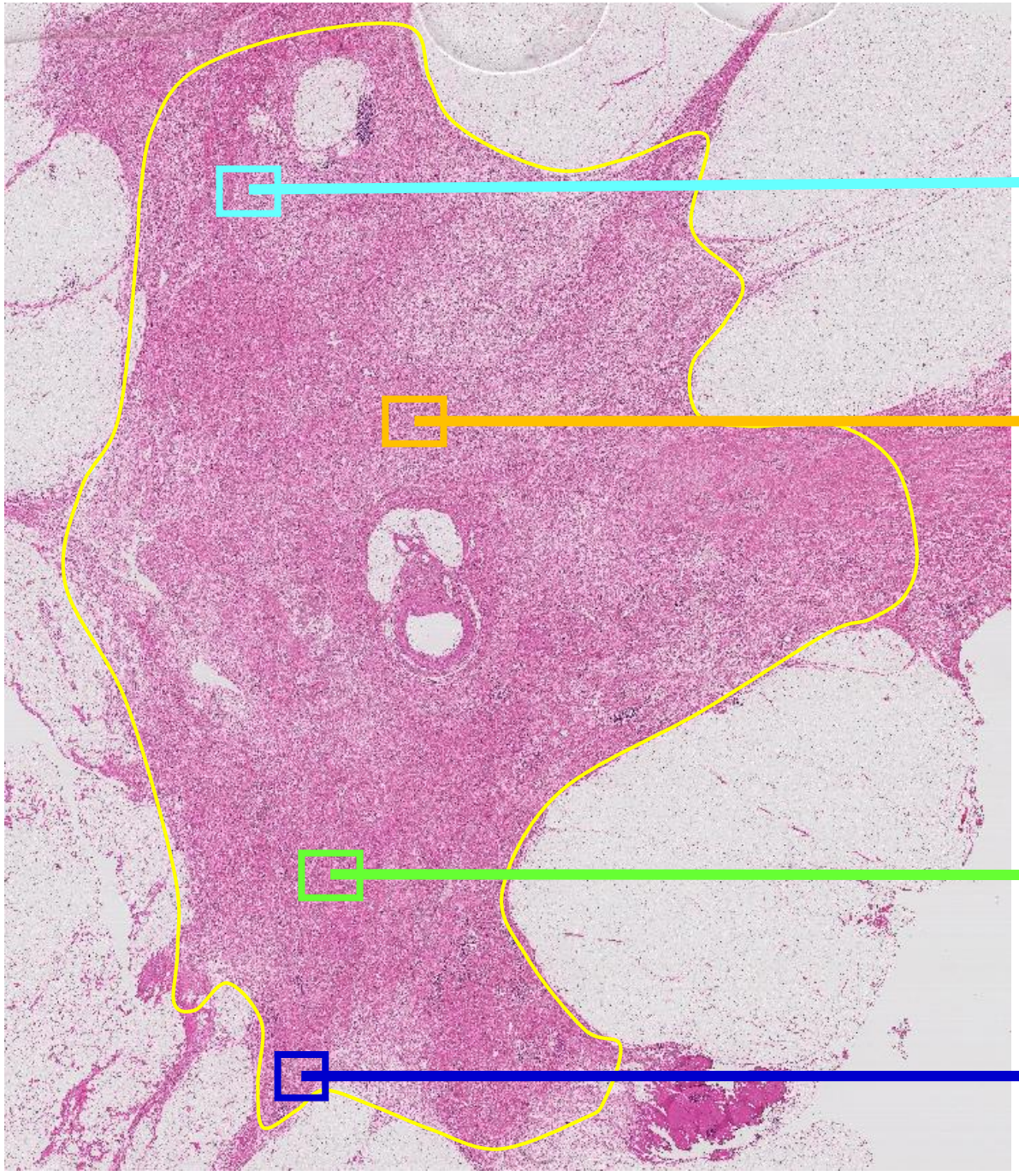
- If the residual tumor bed in case of non-pCR and the area of regression in pCR is larger than 2 cm more slides need to be assessed, with one slide for each further cm of tumor bed as a minimum. For example, if the largest diameter is > 5 cm, then at least 5 representative slides from the largest cross-sectional area should be considered.

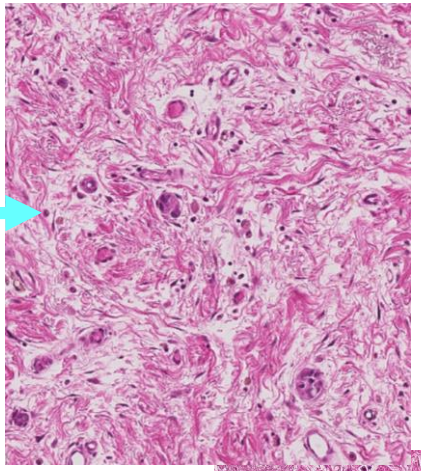
1. Identify area for TIL evaluation

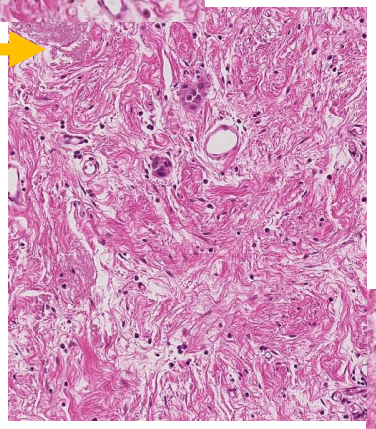


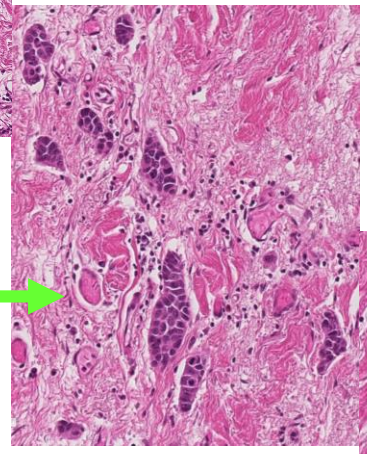
RESIDUAL TUMOR BED - Example 1

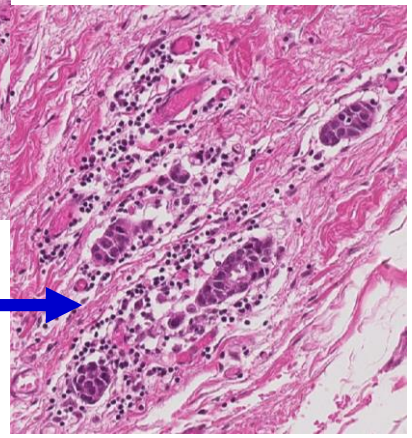
- **Concentric tumor shrinkage, high cellularity. Area of residual tumor bed in yellow.**









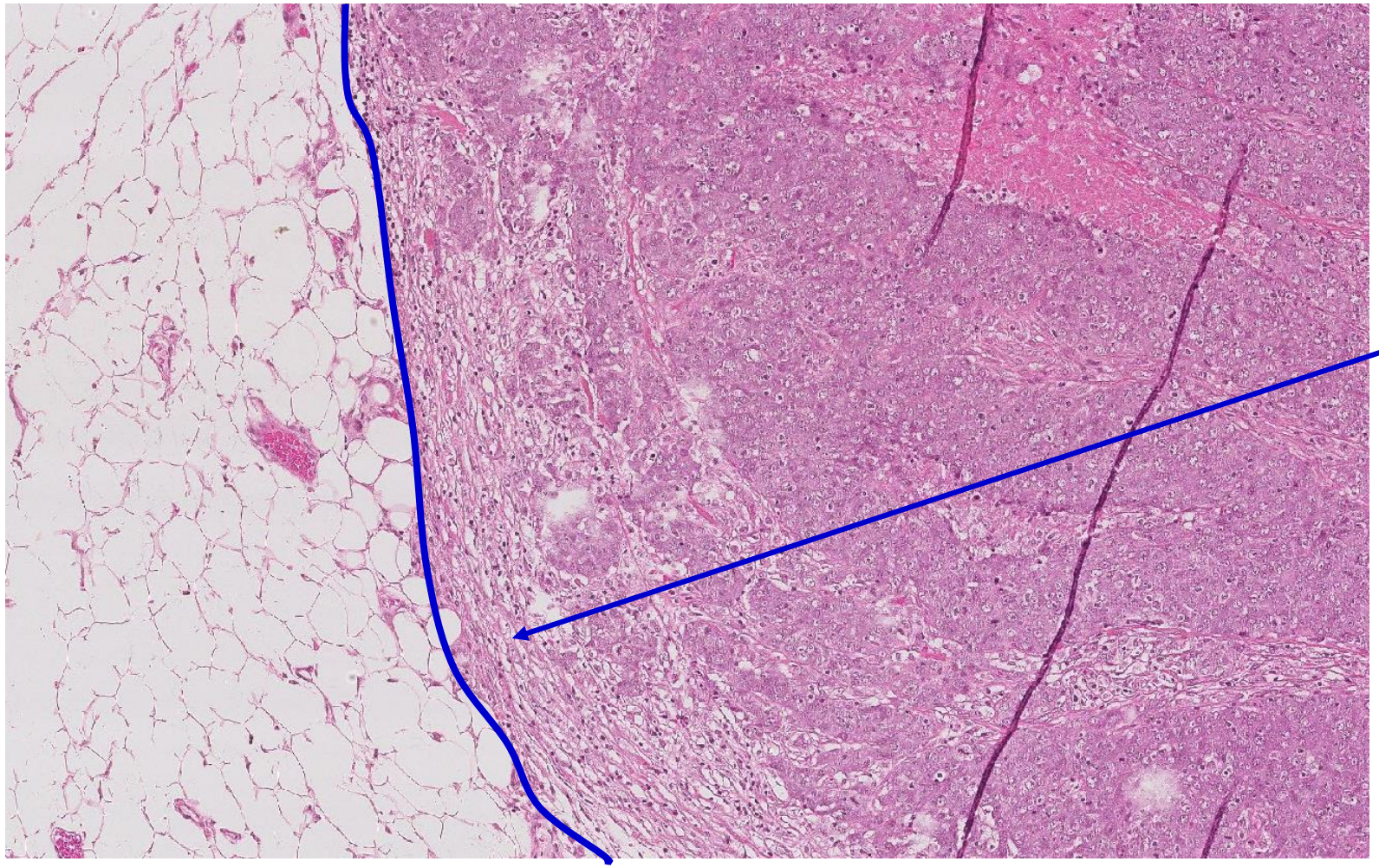


RESIDUAL TUMOR BED - Example 2:

- low cellularity, no concentric shrinkage. Area of residual tumor bed in yellow.

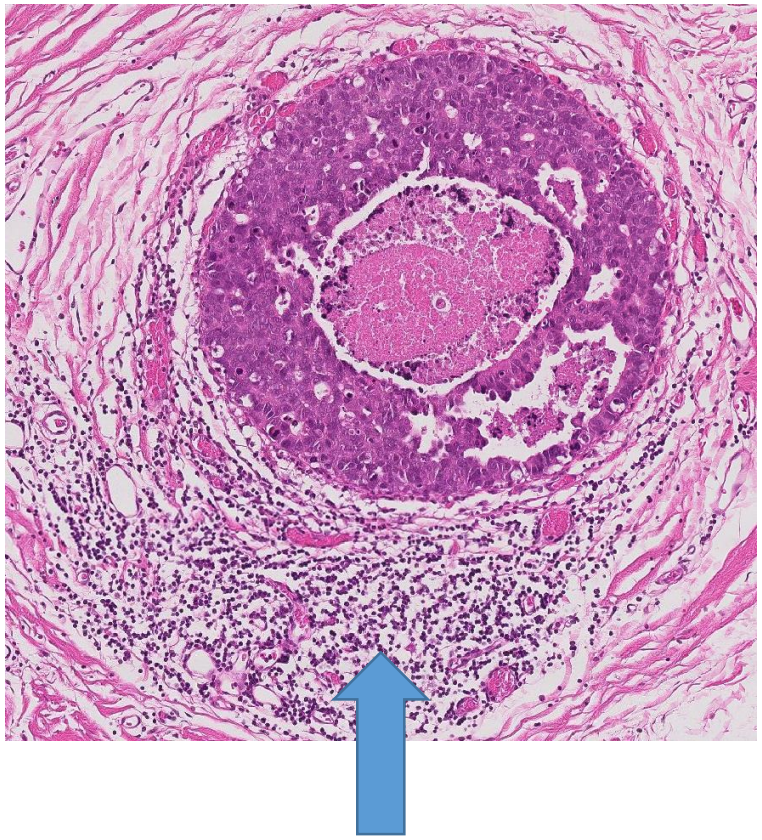
- heterogeneity of TILs around scattered tumor foci and dispersed TILs infiltrates in the tumor bed stroma.

2. Identify the area for TIL evaluation

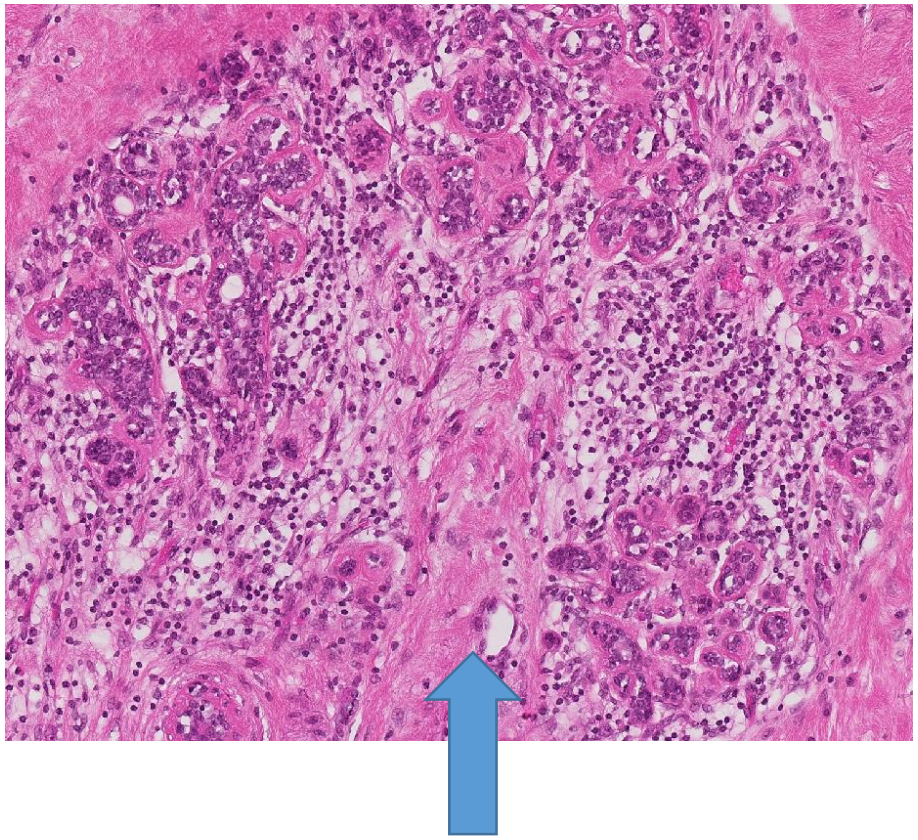


Include TILs immediately adjacent to the tumor border

3. Areas of the tumor bed to be excluded from TIL evaluation



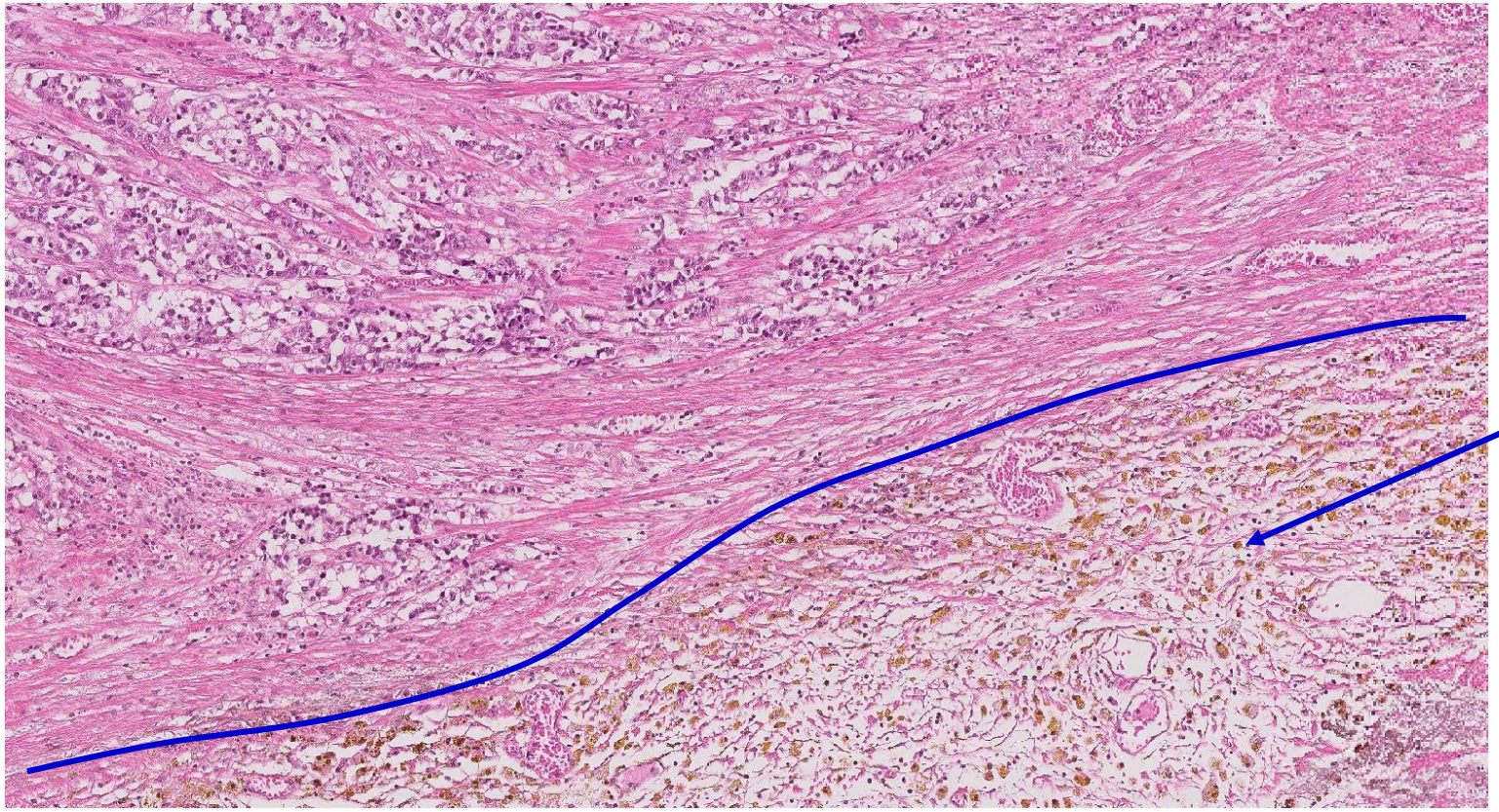
TILs associated with carcinoma in situ



TILs associated with normal lobules

Exclude TILs closely related to remaining foci of carcinoma in situ or normal lobules within the residual tumor bed.

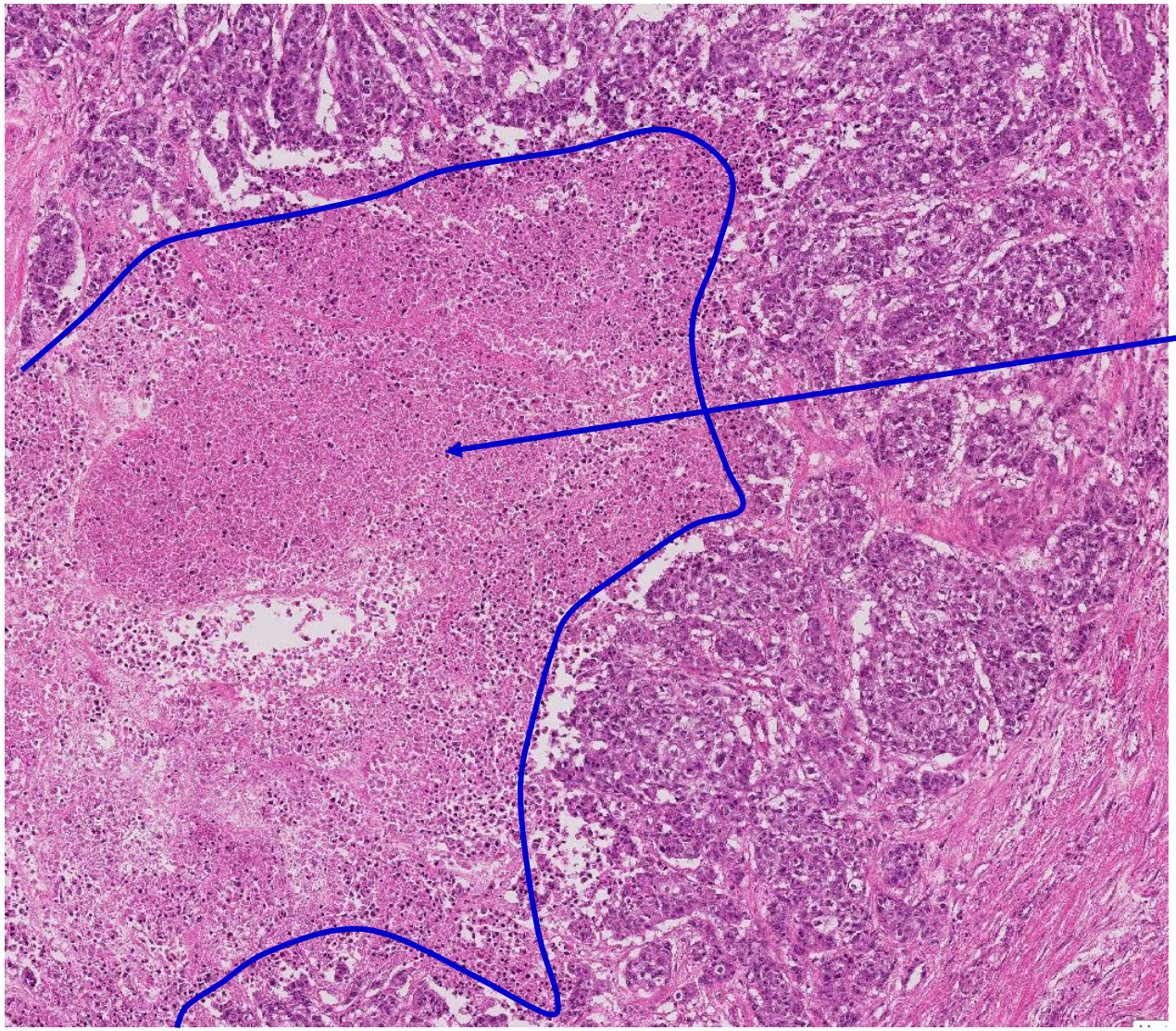
3. Areas of the tumor bed to be excluded from TIL evaluation



Exclude TILs associated with hyalinized and/or edematous vascularized stroma infiltrated by sheets of foamy or hemosiderin-loaded histiocytes.

EXCEPTION: Assess TILs when tumor cells are embedded within aggeragates of histiocytes.

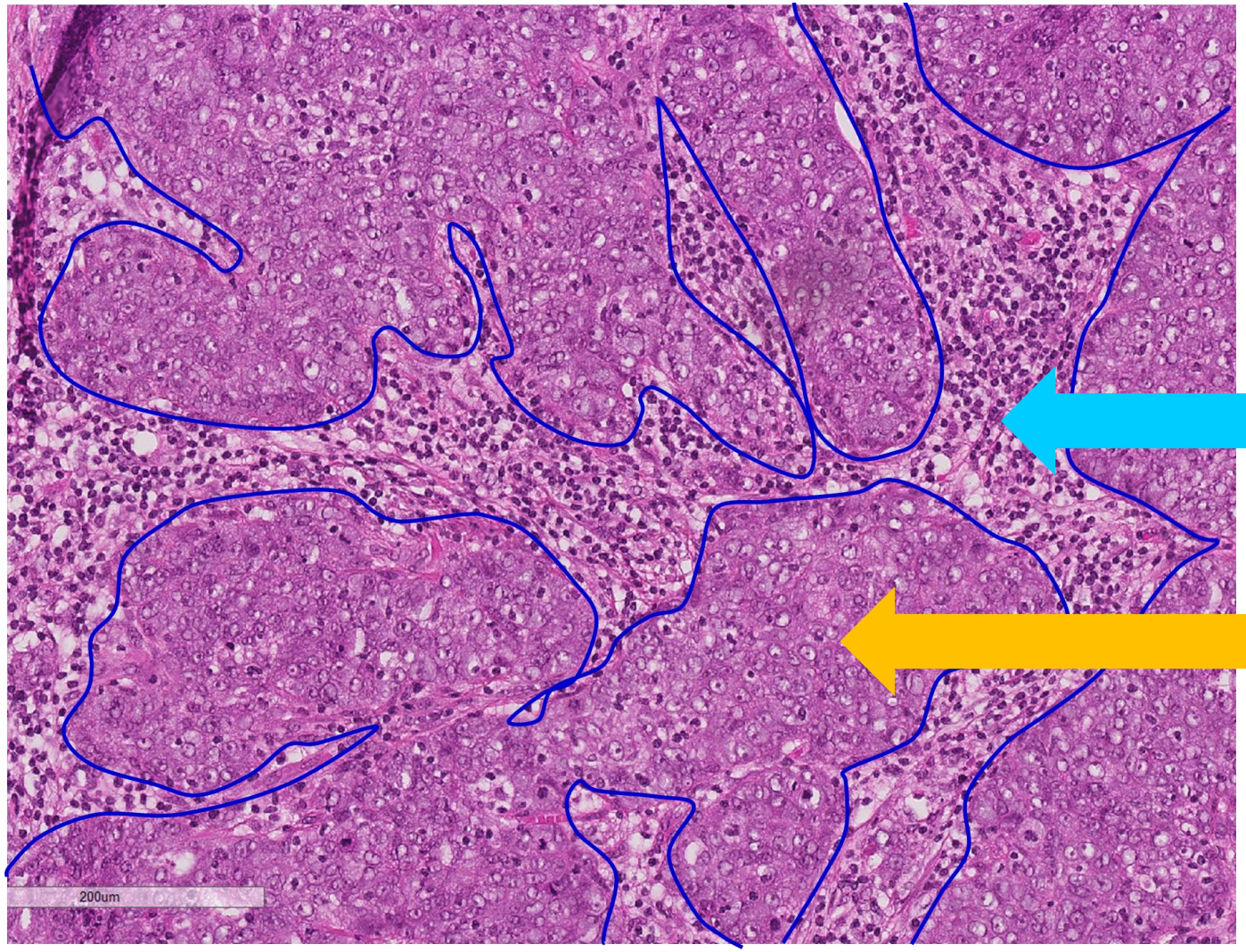
3. Areas of the tumor bed to be excluded from TIL evaluation



Exclude TILs associated with necrotic areas.

Exclude TILs in tumor zones with crush artefacts.

4. Stromal and tumor compartments



TILs should be reported separately mainly for the stromal compartment and, only in particular research settings, the tumor cell compartment.

Stromal TILs %: area occupied by mononuclear inflammatory cells over total stromal area.

Intratumoral TILs %: area occupied by mononuclear inflammatory cells over total tumor cell area.

5. Assess TILs % as continuous parameter

- TILs may provide more biological relevant information when scored as a continuous variable. In daily practice most pathologists will round up to the nearest 5-10%.

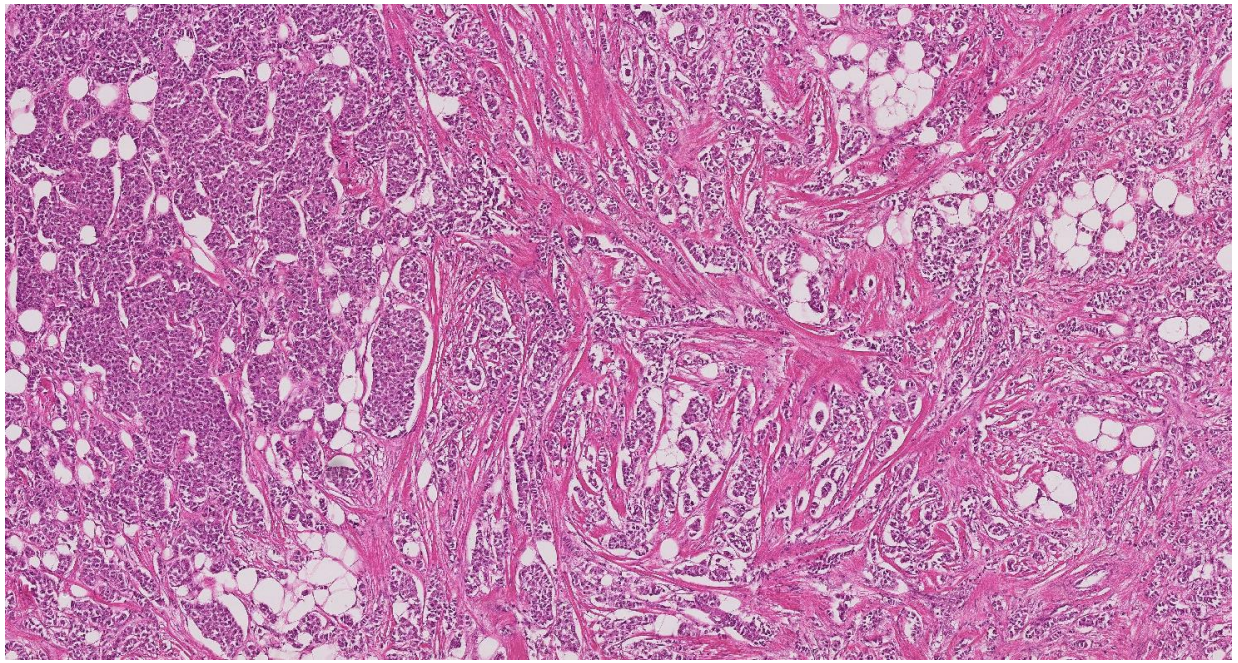
- The percentage of stromal or intratumoral TILs is a semiquantitative parameter. For example, 80% stromal TILs means that 80% of the stromal area shows a dense mononuclear infiltrate. For assessment of percentage values, the dissociated growth pattern of lymphocytes needs to be taken into account. Lymphocytes typically do not form solid cellular aggregates, therefore the designation “100% stromal TILs” would still allow some empty tissue space between the individual lymphocytes.

- Do not focus on hotspots.

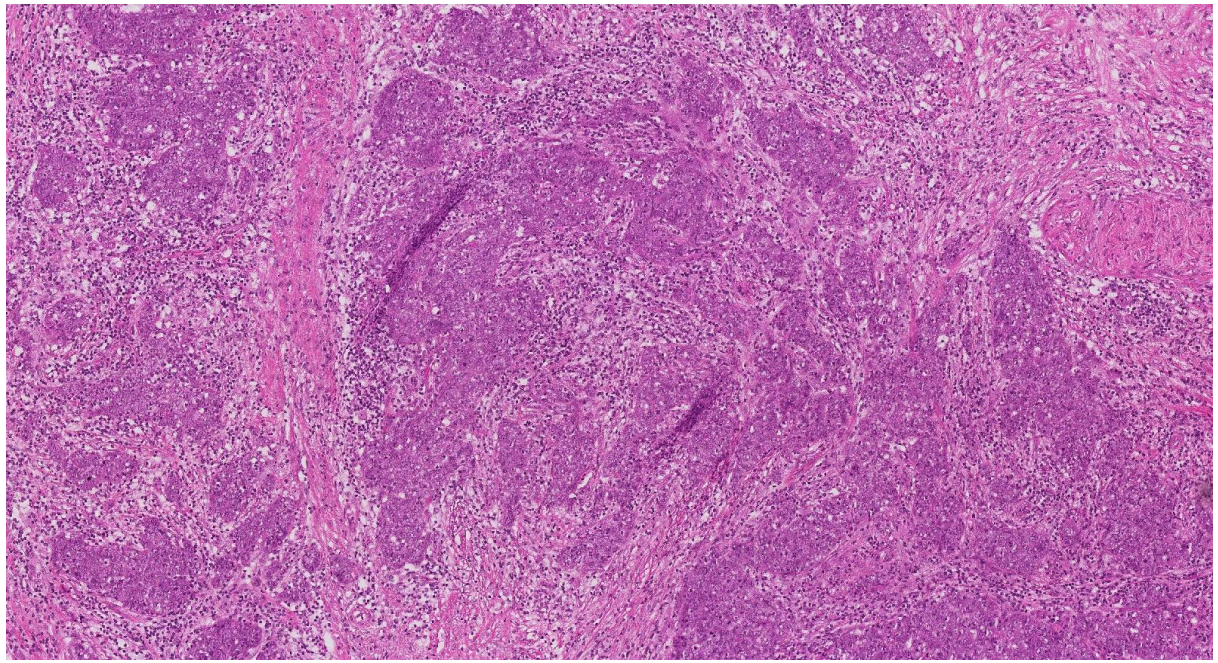
5. Assess TILs % as continuous parameter

Scan across the tumor bed at 50-100x magnification

Example 1

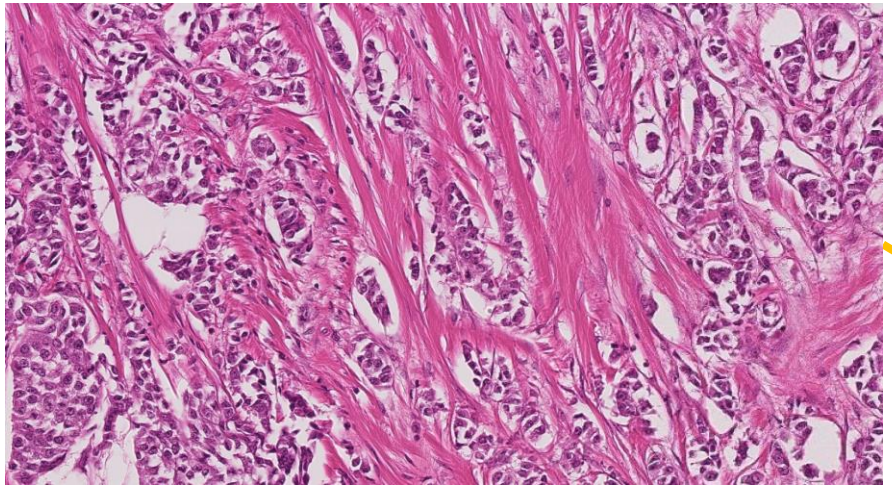


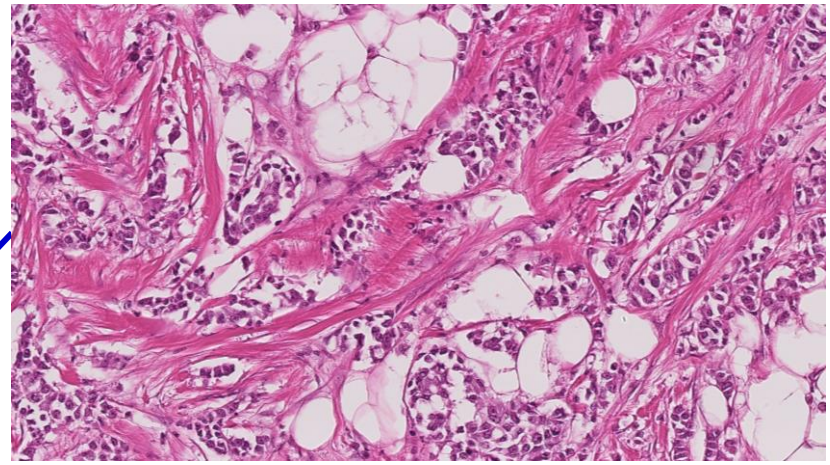
Example 2



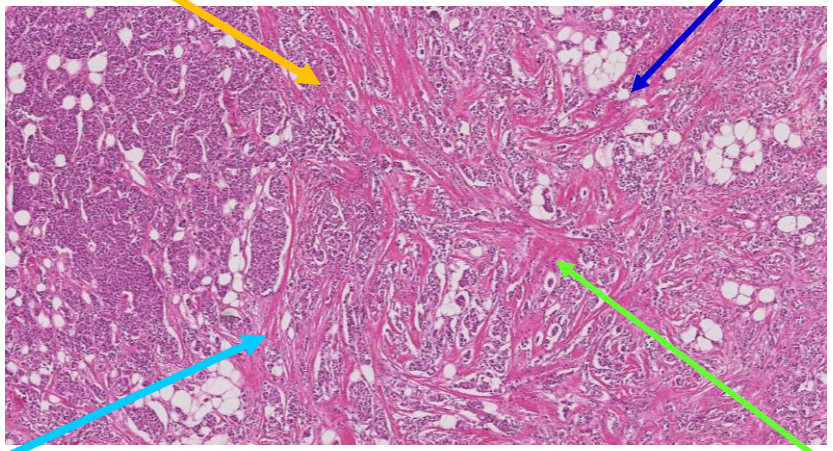
5. Assess TILs % as continuous parameter

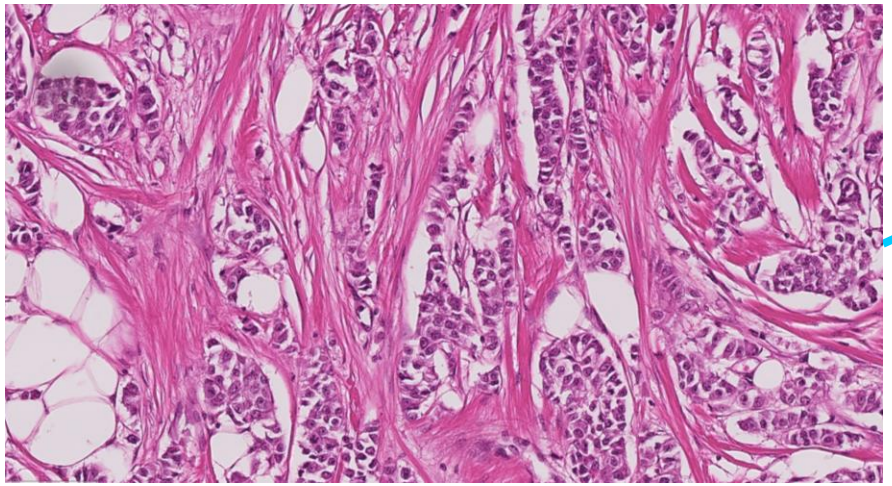
Estimate average TIL from the different microscopic fields (200-400x magnification)

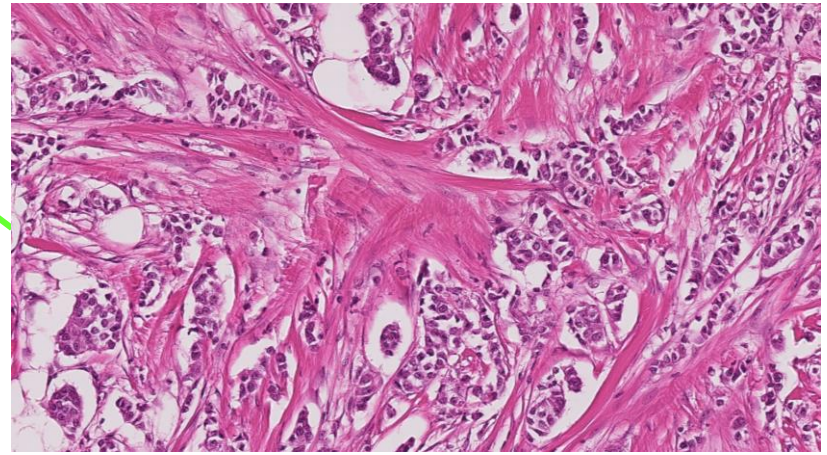




Example 1



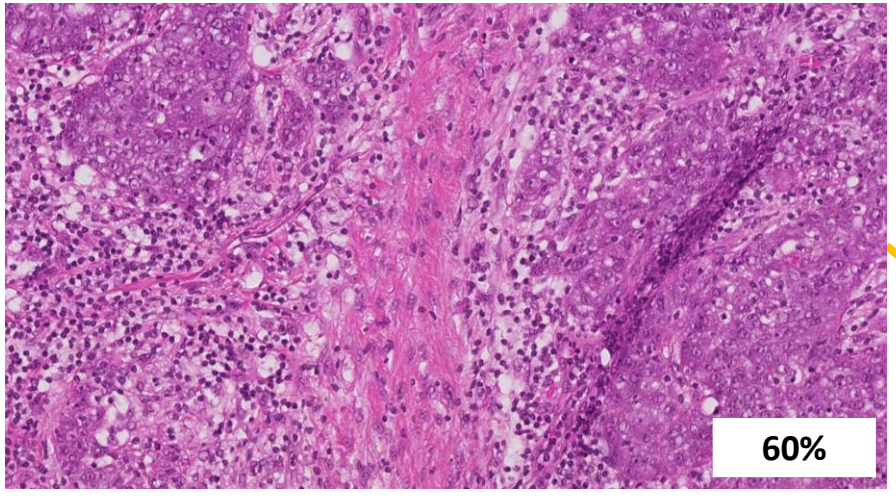




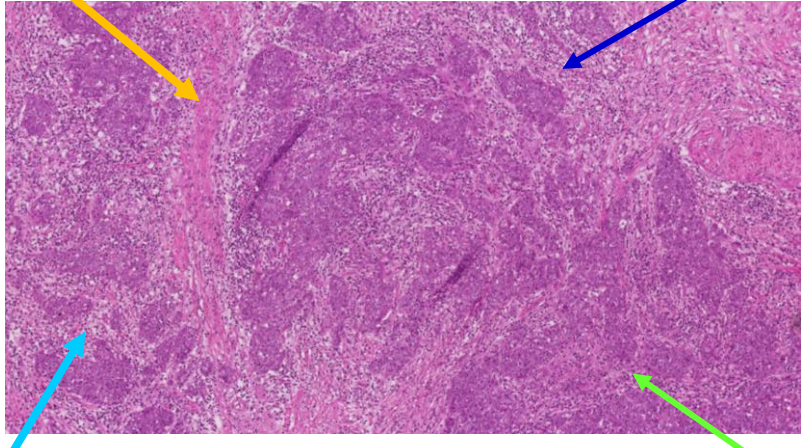
Case with low-TILs, no heterogeneity across fields (<1% in all fields)

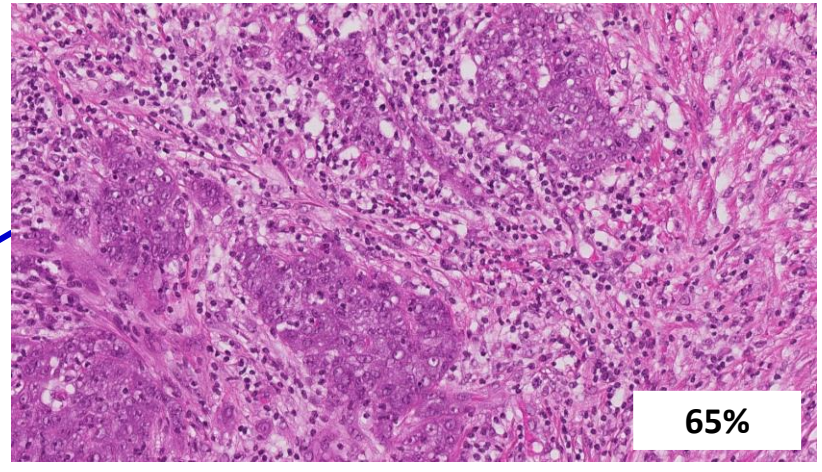
5. Assess TILs % as continuous parameter

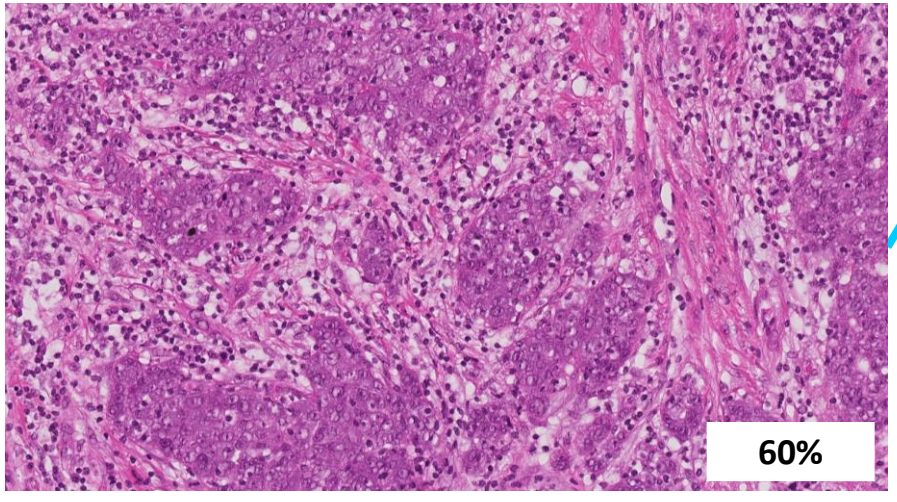
Estimate average TIL from the different microscopic fields (200-400x magnification)



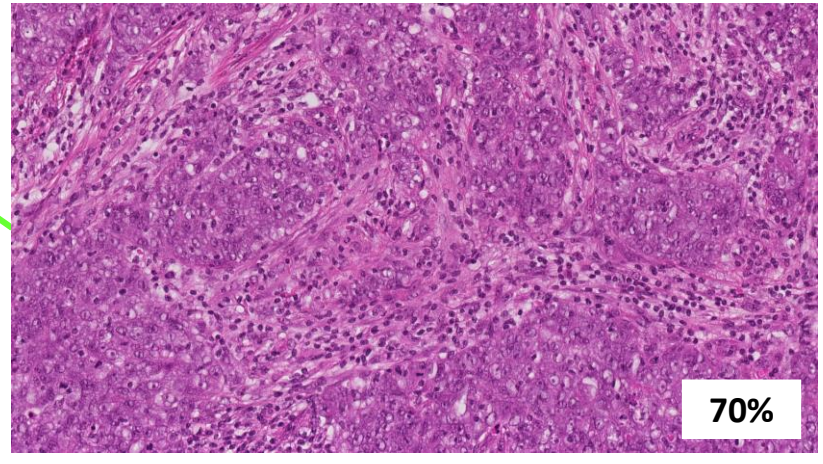
Example 2





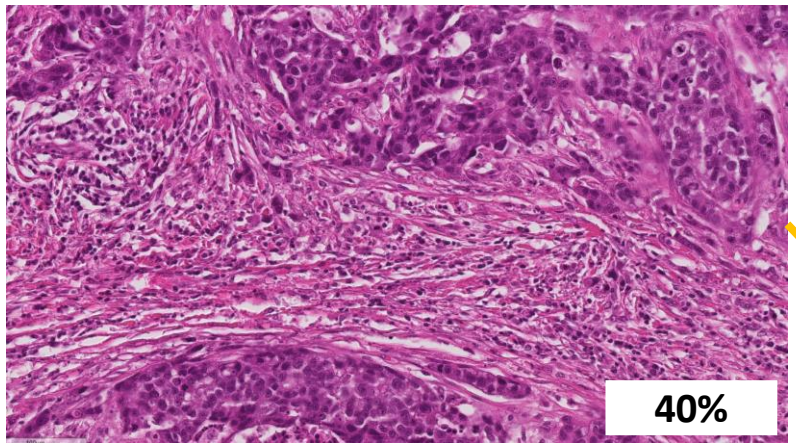


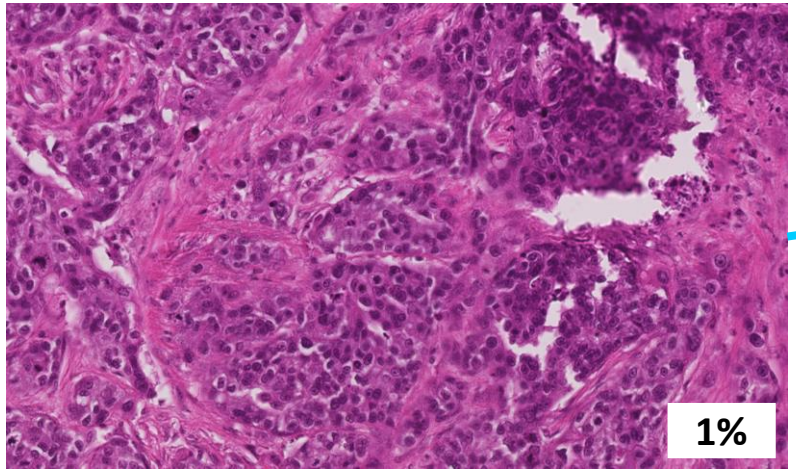
Case with high TILs (>60%), with slight heterogeneity across fields



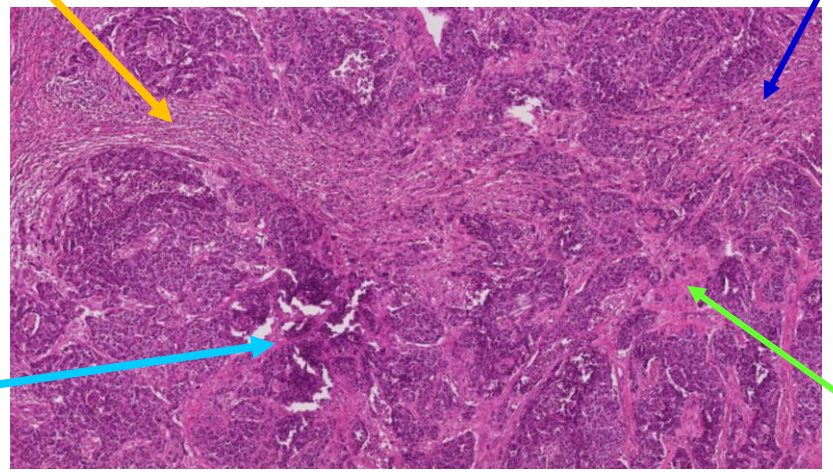
5. Assess TILs % as continuous parameter

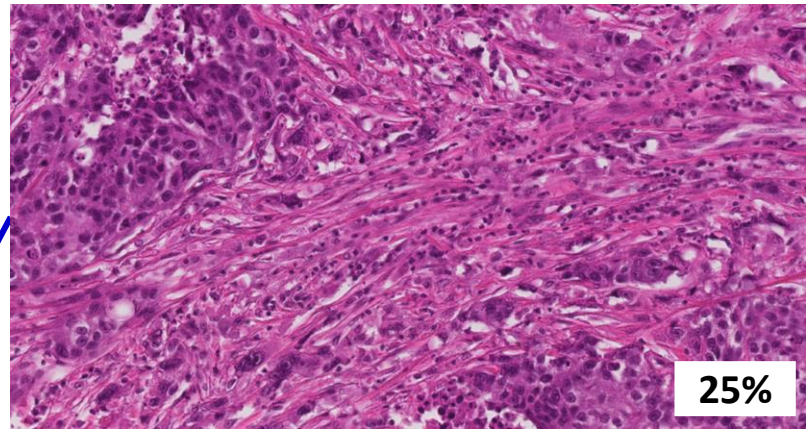
Estimate average TIL from the different microscopic fields (200-400x magnification)

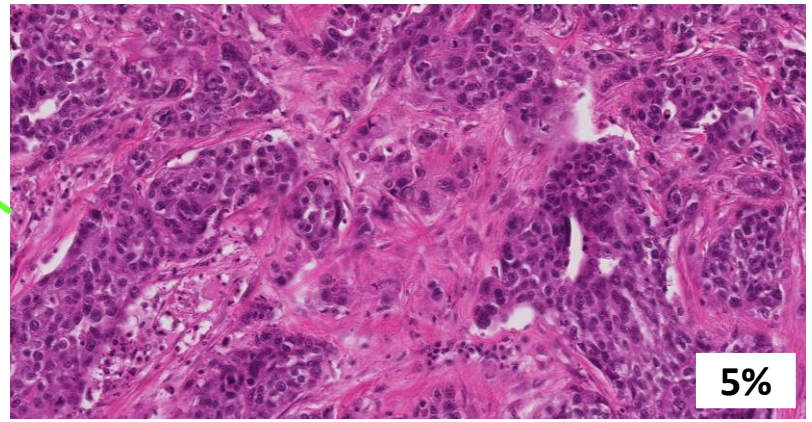




Example 3



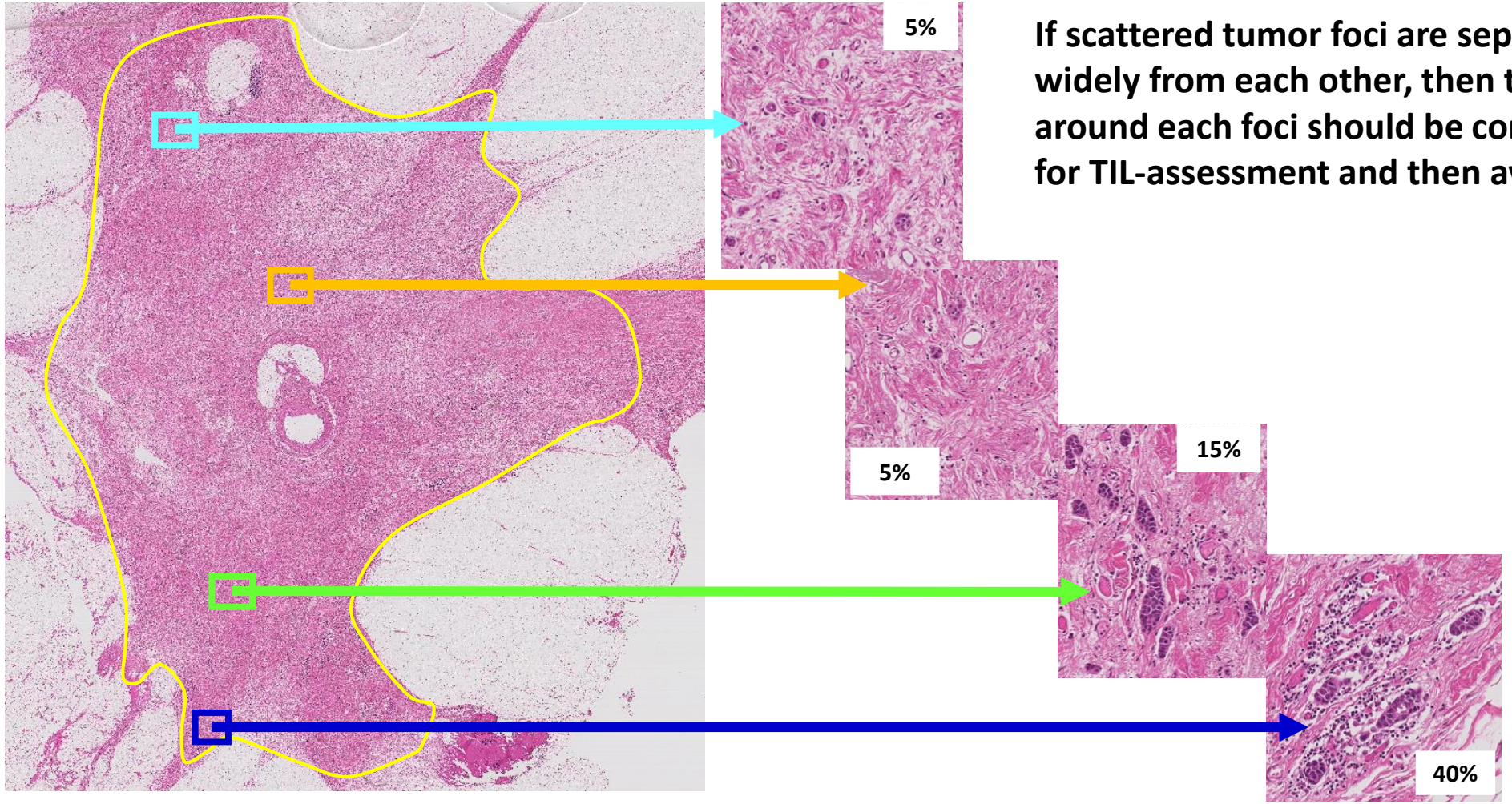




Case with intermediate TILs (20%-40%), with heterogeneity across fields

5. Assess TILs % as continuous parameter

Estimate average TIL from the different microscopic fields (200-400x magnification)



If scattered tumor foci are separated widely from each other, then the area around each foci should be considered for TIL-assessment and then averaged.

For questions please contact:

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