

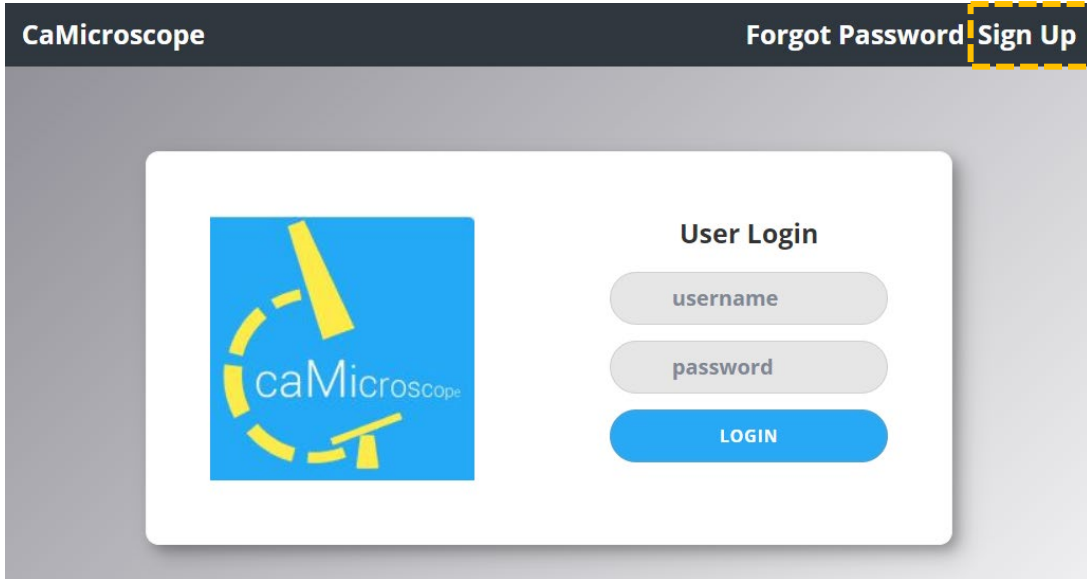
caMicroscope Process Guide

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Create your caMicroscope Account

1. In order to participate in Interactive sTILs Training, the HTT project studies, or ROI Selection on caMicroscope, you will first need to sign up for an account. Go to <https://wolf.cci.emory.edu/camic/htt/> and click "Sign Up."



2. Please read and accept the Consent Form.

Consent Form

The study does not involve any benefits beyond the advancement of science and public health.

Are there risks to me if I participate in this study?
The study does not involve any risks beyond normal working conditions.

My rights as a research participant
Participation in this study is voluntary. There is no compensation for your participation. You have the right to choose not to participate in this study. You may leave the study at any time. Deciding not to participate or choosing to leave the study will not result in any penalty or loss of benefits to which you are entitled.

Page 1 of 2

Confidentiality
All personally identifying information will be kept confidential but may be inspected by a federal entity or federal regulators. Upon enrollment, you will be assigned a participant ID that will link to your real name by a look-up table. This look-up table will only be used by the PI for follow-up purposes, and will otherwise be kept confidential by the PI. Participant consent may be collected electronically or by hard copy. Hard documents will be stored in locked cabinets by the PI through the end of the study. Deidentified data will be shared publicly. Any publications presenting data and results will not be linked to your identity.

What if I have questions?

I Accept The Terms of Consent Form

Agree

[Download Consent Form](#)

Decline

3. Please complete the Basic Information, Professional, and Certifications forms as appropriate for you. Once the required sections are filled, you can click “Next” and/or “Submit” to move onto the next screen. Screen shots provided below.
 - a. After entering all the information and hitting submit, the screen may remain unchanged, but the request is being processed. If you click the submit button a second time, you will get an error message because the account is already made.



Basic Information

First Name *(required)*

Last Name *(required)*

Contact Email *(required)*

Phone Number

Previous

Next



Professional

Institution of Employment *(required)*

Role at your institution *(required)*

- Pathologist
- Resident
- Medical Director
- Other

Previous

Next



Certifications

Board certifications and specialties or equivalent for your country (or enter NA) *(required)*

Board certification or residency organization and country (or enter NA) *(required)*

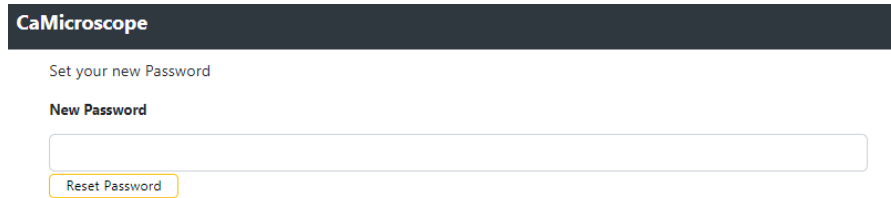
If you are a board-certified anatomic pathologist or the equivalent for your country, enter the number of years since your certification. (If you are not a board certified anatomic pathologist or equivalent, enter -1) *(required)*

If you are an anatomic pathology resident, how many years of residency have you had? (If you are not an anatomic pathology resident, enter -1) *(required)*

Previous

Submit

4. Within a few minutes, an email will be sent to the address you registered with (Subject: "caMicroscope : Welcome" from rbirmin@emory.edu). Please make sure to click the "Reset your password" link to be directed to a page to create a password for your account. The link will expire in one hour. In this case visit the login page and click "Forgot Password" in the top-right corner and another email with a link to reset your password will be sent.



The screenshot shows a web interface for resetting a password. At the top, there is a dark header with the text "CaMicroscope" in white. Below the header, the text "Set your new Password" is displayed. Underneath, the label "New Password" is positioned above a single-line text input field. Below the input field, there is a yellow button with the text "Reset Password".

5. Once a password is created, you can return to <https://wolf.cci.emory.edu/camic/htt/> and log in with your credentials. Your username is your email address.
 - a. The platform starts empty. HTT project management needs to assign you collections.
 - b. Response time to your registration, should be less than two days.
 - c. Please allow five business days for your registration to be processed by the HTT project management and for you to be sent further instructions.
 - d. If you haven't been contacted after five days, please email brandon.gallas@fda.hhs.gov.

How to Access HTT Interactive Training & Pivotal Studies

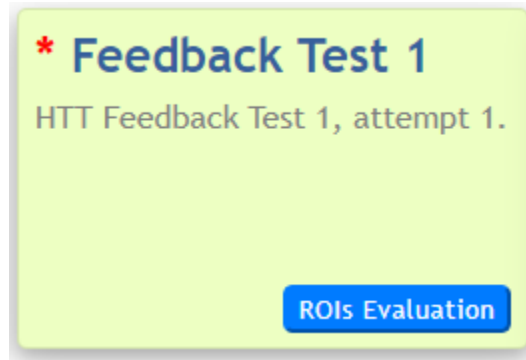
1. Go to <https://wolf.cci.emory.edu/camic/htt/>. Login to your caMicroscope account with the email address and password you used to register. You will be brought to the landing page.

The screenshot shows the CaMicroscope website interface. At the top, there is a dark navigation bar with the text "CaMicroscope" on the left, "hi, victor.garcia.2" in the center, and "FEEDBACK" on the right. Below the navigation bar, the main heading is "MDDT DATA COLLECTION". Underneath, there is a section titled "USCAP Annual Meeting" with the following details: "Feb. 28, 2020", "Los Angeles Covention Center", and "Los Angeles, CA". A large histological image of a tissue section is displayed. Below this image, there are two columns of content. The left column is titled "ROI ANNOTATIONS" and includes the text "Participants randomly pick up a ROI and annotate on it." Below this text is a button labeled "PICK & ANNOTATE", which is highlighted with a red rectangular border. The right column is titled "WHOLE SLIDE IMAGING" and includes the text "Save & Download Our Public WSI". Below this text is a button labeled "DOWNLOAD SLIDES".

2. Click **"Pick & Annotate"** under the **"ROI Annotations –Participants"** module to find the interactive training and pivotal study collections.

This screenshot shows a detailed view of the "ROI ANNOTATION - PARTICIPANTS" module. On the left is a large histological image of a tissue section. To the right of the image, the text reads "ROI ANNOTATION - PARTICIPANTS" followed by "Participants randomly pick up a ROI and annotate on it." Below this text is a button labeled "PICK & ANNOTATE".

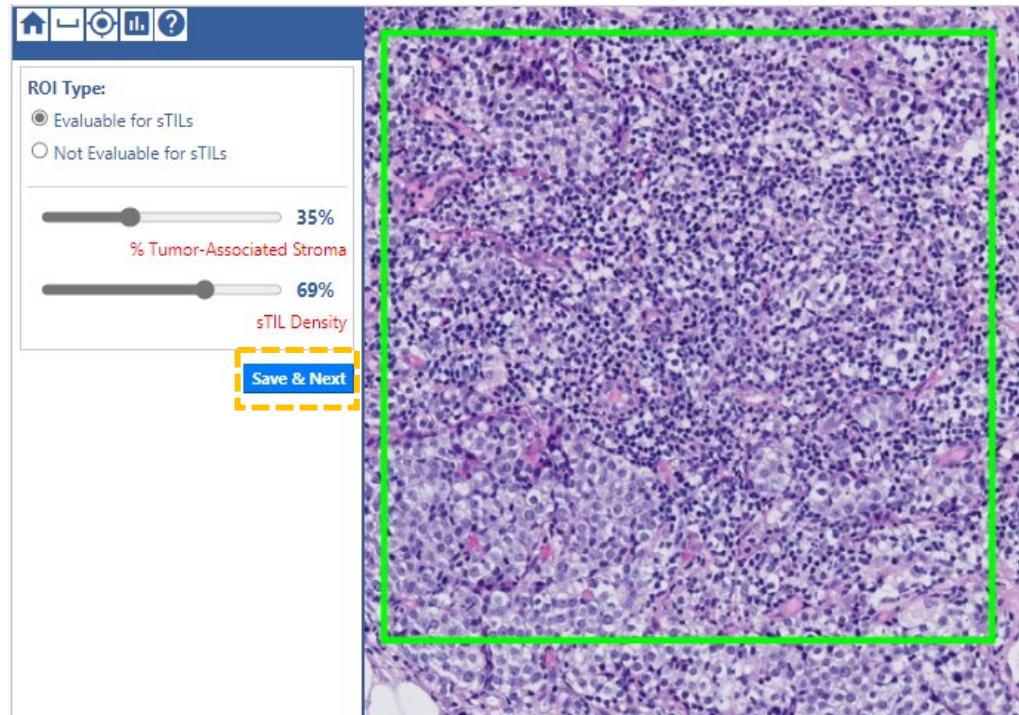
- Click the “ROIs Evaluation” to select the collection you would like to complete (example below: “Feedback Test 1”).



- Once in the collection, click “Open” to begin the sTILs evaluation on the ROIs within a slide. The number of ROIs on a slide may vary and your “ROIs” column will update as you complete the task.

| HOME COLLECTION LIST | | | | | | hi, Victor | SIGN OUT |
|------------------------------|------------------|-----------|----------|---------------------|------|----------------------|----------|
| 50 entries per page | | Search... | | | | | |
| ID | Name | Study | Specimen | MPP | ROIs | | |
| 0100000000000000000000000004 | T01-TILS-001-04B | | | 0.22834699609526637 | 0/1 | Open | |
| 0100000000000000000000000003 | T01-TILS-001-03B | | | 0.22834699609526637 | 0/1 | Open | |
| 0100000000000000000000000029 | T01-TILS-001-29B | | | 0.22834699609526637 | 0/1 | Open | |
| 0100000000000000000000000027 | T01-TILS-001-27B | | | 0.22834699609526637 | 0/2 | Open | |
| 0100000000000000000000000034 | T01-TILS-001-34B | | | 0.22834699609526637 | 0/1 | Open | |
| 0100000000000000000000000042 | T01-TILS-001-42B | | | 0.22834699609526637 | 0/1 | Open | |
| 0100000000000000000000000032 | T01-TILS-001-32B | | | 0.22834699609526637 | 0/1 | Open | |
| 0100000000000000000000000033 | T01-TILS-001-33B | | | 0.22834699609526637 | 0/4 | Open | |
| 0100000000000000000000000057 | T01-TILS-001-57B | | | 0.22834699609526637 | 0/2 | Open | |
| 0100000000000000000000000053 | T01-TILS-001-53B | | | 0.22834699609526637 | 0/1 | Open | |
| 0100000000000000000000000050 | T01-TILS-001-50B | | | 0.22834699609526637 | 0/1 | Open | |

5. Annotate the ROI presented, then click “Save & Next” to save your annotation and move to the next ROI. It may take a moment to move to the next ROI. When you finish annotating all ROIs for a WSI, you will return to the list of cases in step 4. Notes on the annotation:
- ROI Type: If the ROI is within 1 mm of the tumor (500 μm of the tumor margin) and includes stroma, it is “Evaluable for sTILs”. Otherwise, it is not.
 - % Tumor-Associated Stroma: The area of tumor-associated stroma divided by the area of the ROI.
 - sTILs Density: The area of the tumor-infiltrating lymphocytes divided by the area of the tumor-associated stroma.



- d. If you are annotating the “Feedback Test”, you will see feedback from the experts (scores, comments and pitfalls) before moving to the next ROI. Select “Next” to continue.

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?

ROI Type:

Evaluable for sTILs

Not Evaluable for sTILs

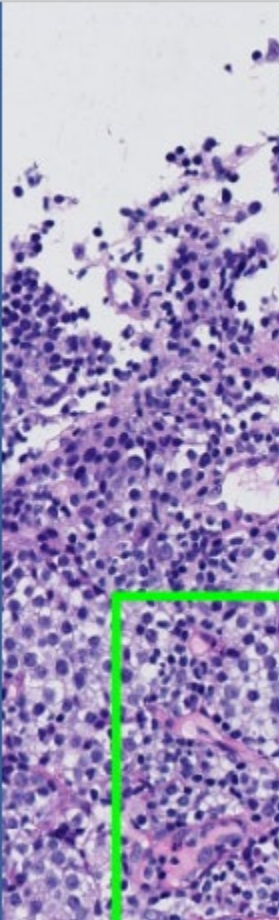
Expert Panel Annotations:

| ROI Type | % Tumor-Associated Stroma | % sTIL Density |
|-----------|---------------------------|----------------|
| Evaluable | 30 | 90 |
| Evaluable | 60 | 95 |
| Evaluable | 50 | 92 |
| Evaluable | 50 | 75 |
| Evaluable | 60 | 90 |
| Evaluable | 60 | 90 |

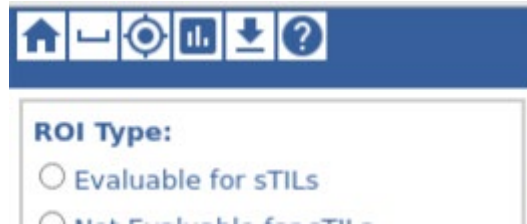
Mean Percent Stroma: 51.7

Mean sTIL Density: 88.7

Comments: A challenging case. The extensive number of lymphocytes obscures the background, and it is not



Overview of caMicroscope Icons



Icon functionality listed in order of left to right:

1. Return to list of assigned WSIs and ROIs (Collection List)
2. Open the measurement ruler
3. Return to the center of the ROI
4. View the TILs cheat sheet
5. Download the WSI (if permitted)
6. View slides of a tutorial presentation on the sTILs assessment

ROI Selection Protocol

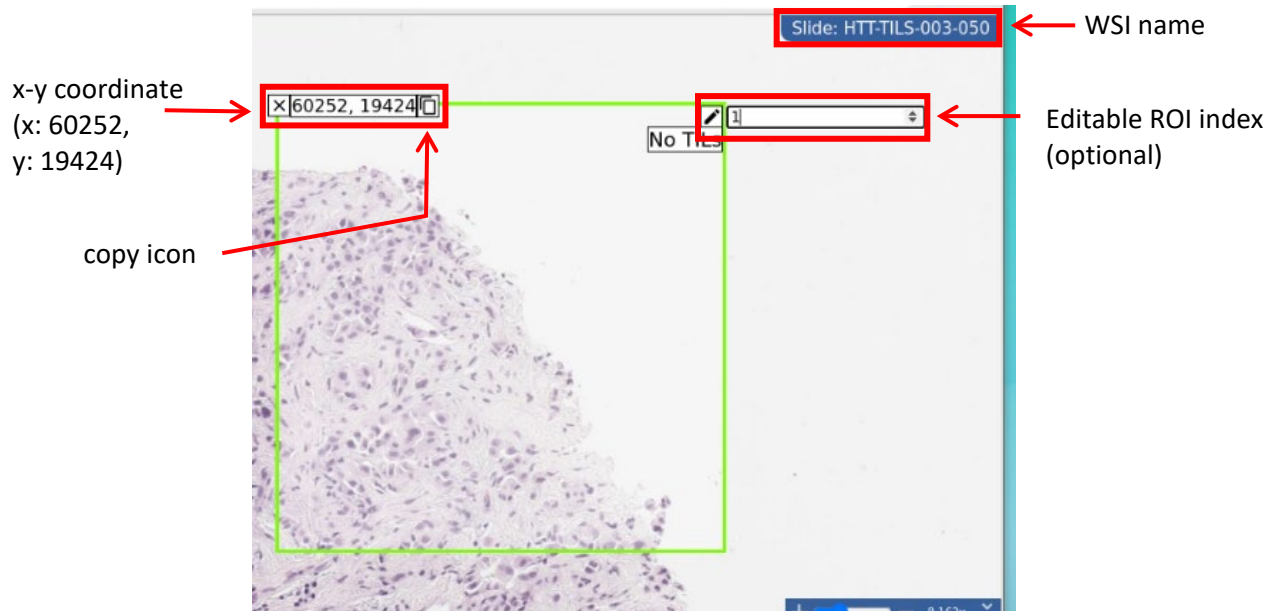
Annotate all WSIs in the assigned collection.

1. 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 of 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](#).

| | |
|--|--|
| 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 |

2. Provide Annotations on selected ROIs in the spreadsheet provided.
 - a. Study Annotations: Evaluable/Not Evaluable; % Tumor-Associated Stroma; sTILs Density
 - b. Targets in each ROI: Record which pitfalls are present in each ROI.

3. Example selected ROI:



- a. Each row of the spreadsheet corresponds to one ROI.
- b. Record the ROI index (ex: 1, 2, 3 ...) for each ROI on the image viewing platform. (optional)
- c. Record the x-y position on the spreadsheet. The coordinates can be seen on the image viewing platform. Use the "copy" icon to copy to your clipboard.
- d. Record the primary study data on the spreadsheet: Evaluable/Not Evaluable; % Tumor-Associated Stroma; % sTILs
- e. Record the identified targets from among the ROIs. The spread sheet will show a running total.

Summary of the Five Steps of the sTILs Assessment

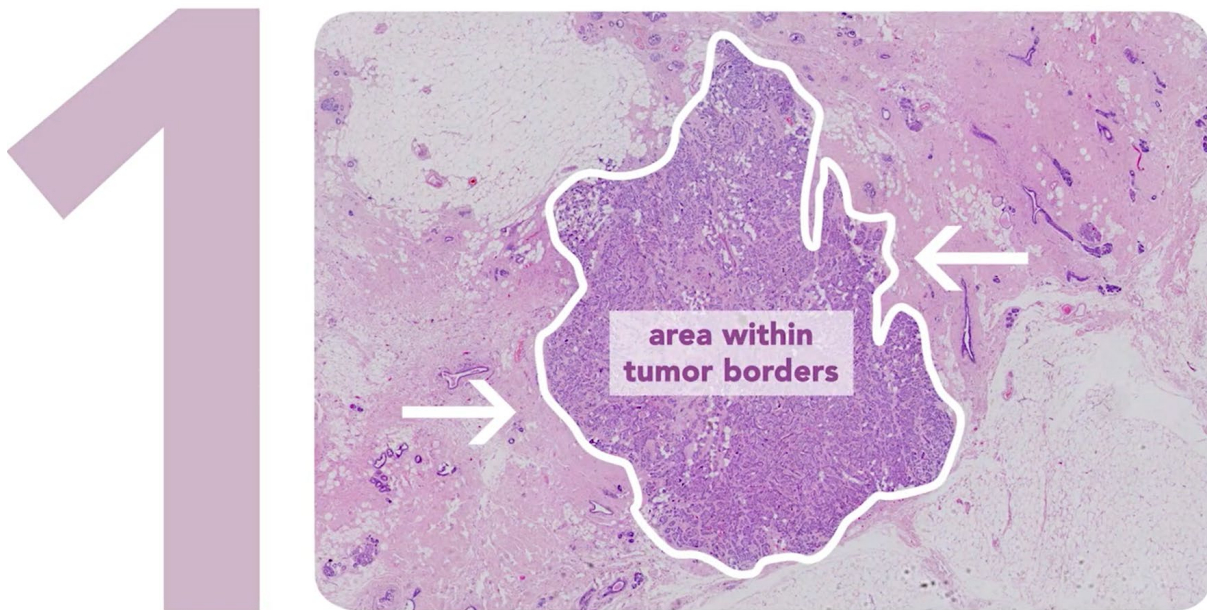
Below are screen shots from the training video that was part of the FDA CME course, "Assessment of Stromal Tumor-Infiltrating Lymphocytes."

- Link to the video on YouTube: <https://www.youtube.com/watch?v=aPa-pXIBBIU>

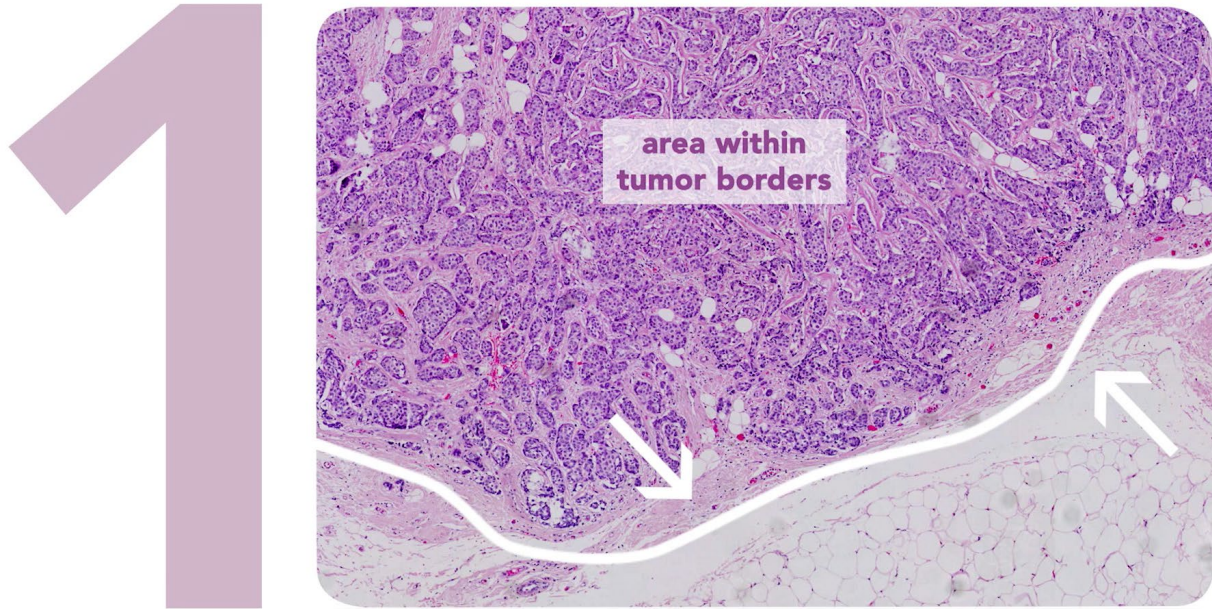
5 steps



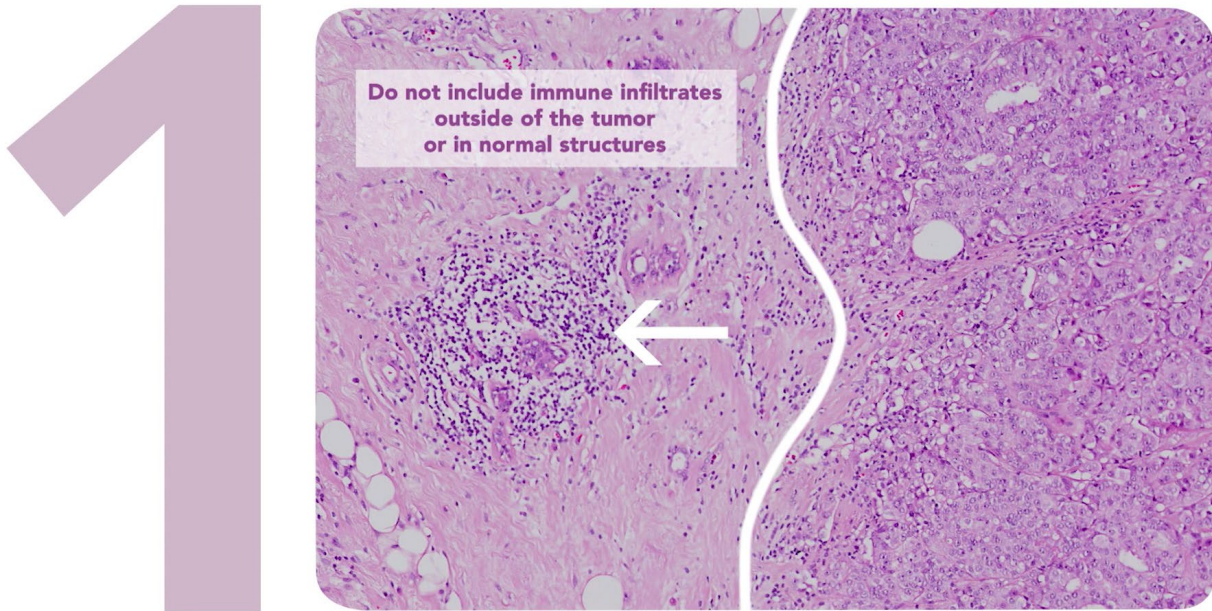
1. Define the areas for TILs evaluation
 - a. Only TILs within the borders of invasive tumors are evaluated



b. The invasive edge is included in the evaluation, but not reported separately

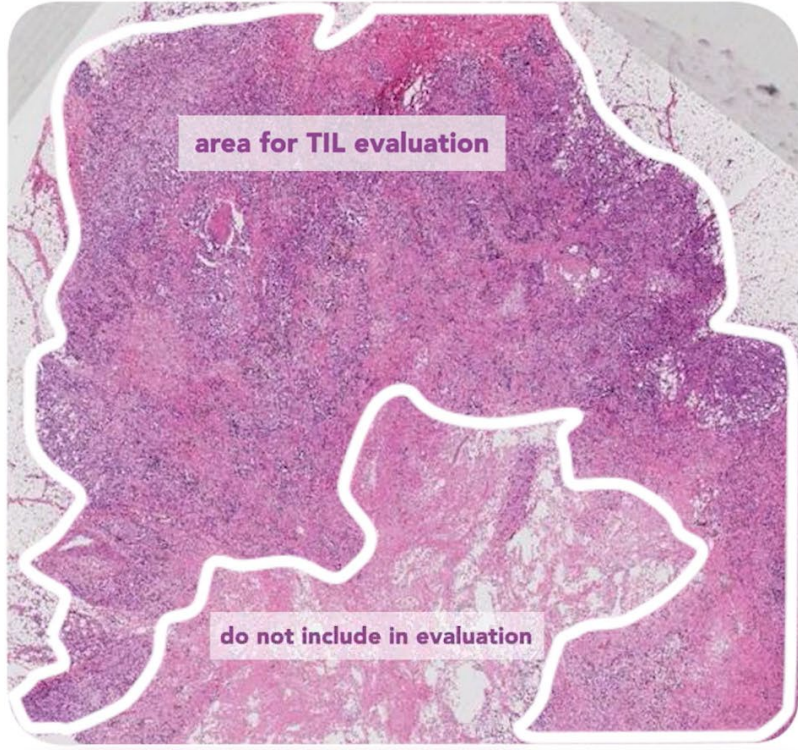


c. Immune infiltrates outside the tumor borders are not included



d. Large areas of central necrosis or fibrosis are not included in the evaluation

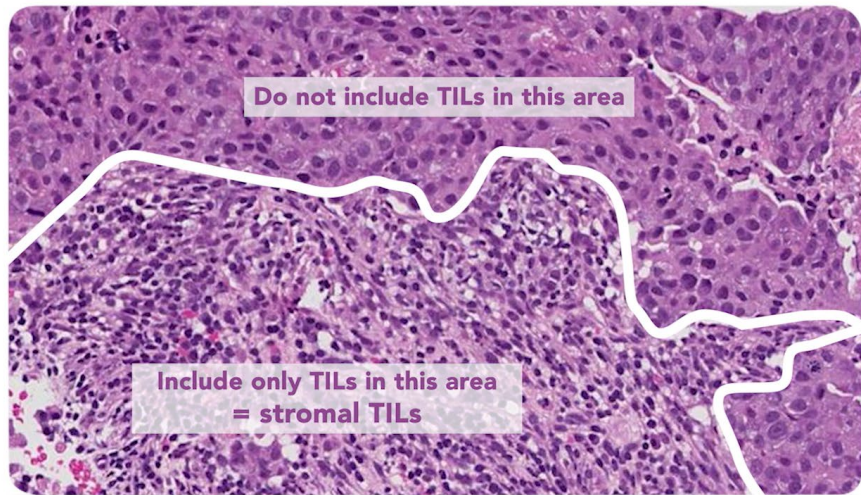
1



2. Focus on stromal TILs

- In the clinical setting, only stromal TILs are relevant.
- Scoring TILs in the intratumoral compartment is poorly reproducible between pathologists, that's why we only score TILs in the stromal area

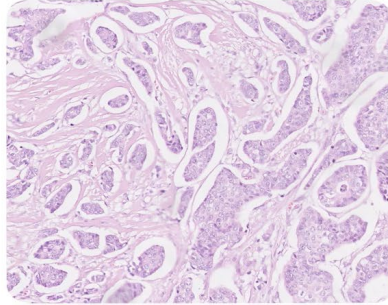
2



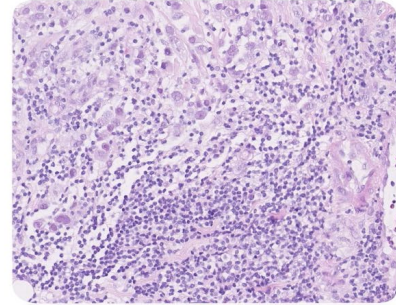
- Scan the tumor at low magnification and focus on tumor stroma

2

Stroma contains predominantly collagenous tissue, few immune cells



Stroma contains predominantly immune cell infiltrate, collagenous tissue difficult to recognize

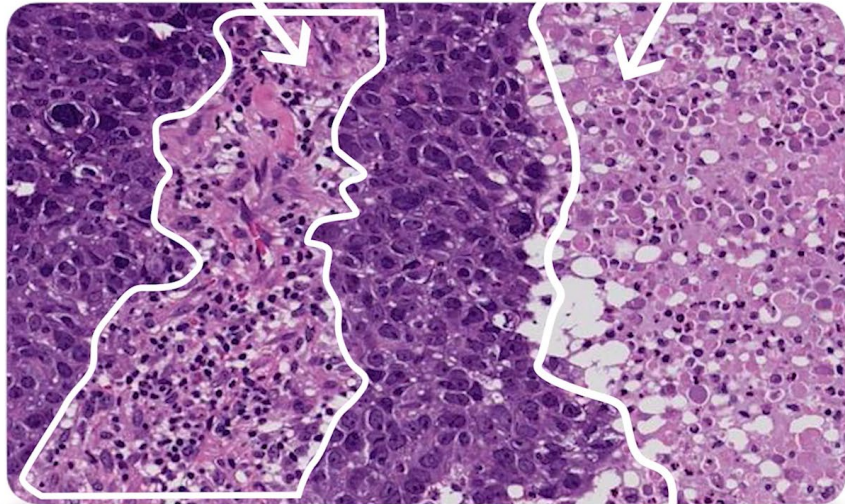


3. Determine the type of inflammatory infiltrate
 - a. Include only mononuclear cells, lymphocytes and plasmacytes.
 - b. Do not include granulocytes or immune cells in necrotic areas.

3

Mononuclear stromal TILs infiltrate

Do not include immune cells in necrotic areas



4. As a first approach, include tumor in one of three groups based on low magnifications and assess % stromal TILs
 - a. The denominator used to determine the percentage stromal TILs is the area of stromal tissue, not the number of stromal cells

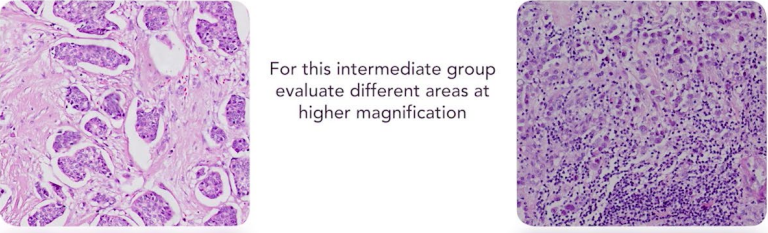
4

Group A
tumor with no/minimal immune cells
0-10% stromal TILs

Group B
tumor with intermediate / heterogeneous infiltrate
10-40% stromal TILs

Group C
tumor with high immune infiltrate
40-90% stromal TILs

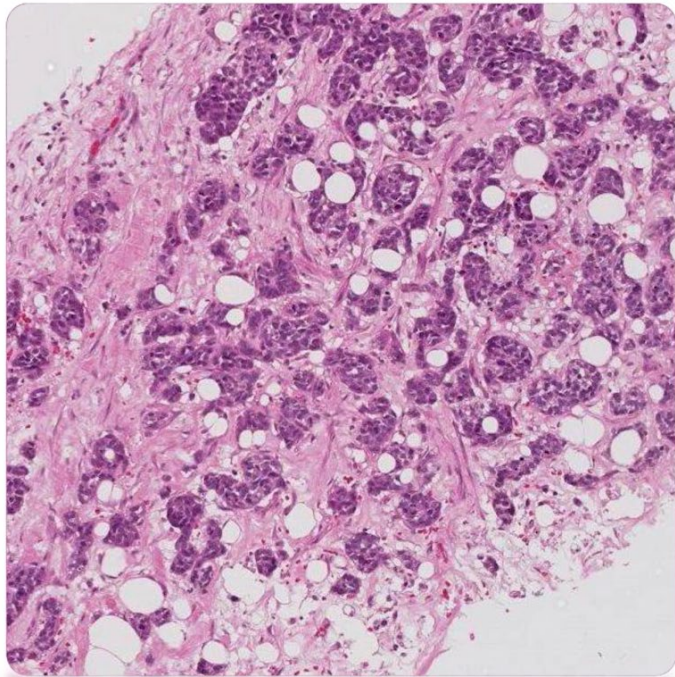
For this intermediate group evaluate different areas at higher magnification



5. Report the percentage of TILs
 - a. Report the average of the stromal areas you see, don't focus on hotspots

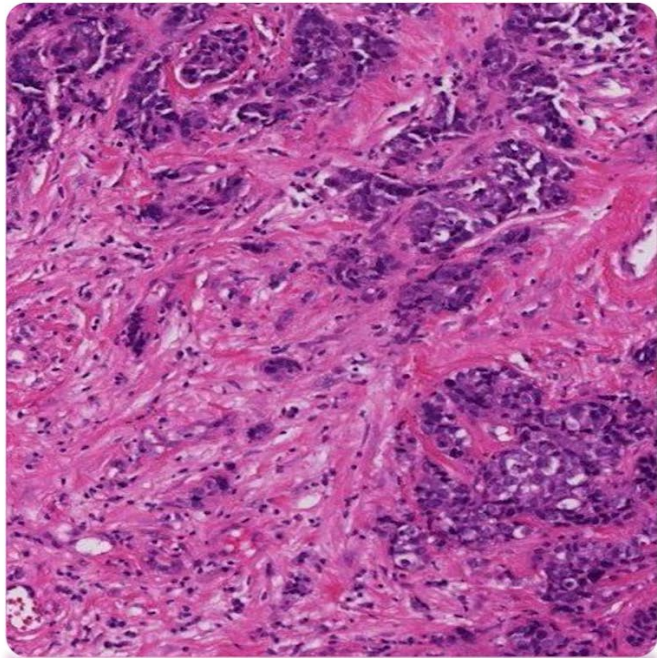
TILs score = 0 %

5



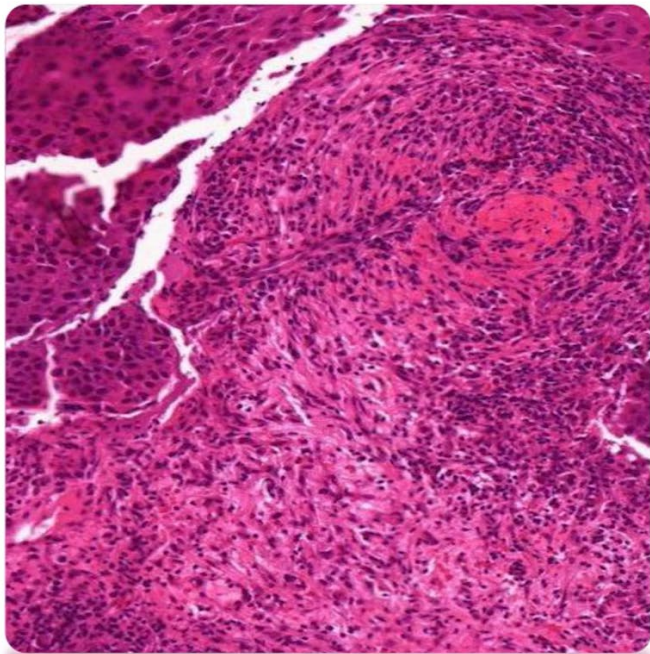
5

TILs score = around 10%



5

TILs score = around 50%



5

TILs score > 80%

