

# Development of Pathologist Training Materials using Consensus Driven Annotations of sTILs Assessment in Breast Cancer

Victor L. Garcia, MD

ORISE Fellow

U.S. Food and Drug Administration  
Office of Science and Engineering Laboratories

Center for Devices and Radiological Health  
Division of Imaging, Diagnostics, Software Reliability

**Pathology  
Informatics  
Summit 2022**

David L. Lawrence Convention Center  
The Westin Pittsburgh



# Co-Authors

- **Katherine Elfer, PhD MPH**
  - FDA/CDRH/OSEL/ Division of Imaging, Diagnostics, and Software Reliability
  - NIH/NCI/Division of Cancer Prevention/Cancer Prevention Fellowship Program, Bethesda, MD
- **Dieter J.E. Peeters, MD PhD**
  - Department of Pathology, Sint-Maarten Hospital, Mechelen, Belgium
  - Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium
  - Histopathology, Imaging and Quantification Unit, CellCarta NV, Antwerp, Belgium
- **Anna Ehinger, MD**
  - Department of Genetics and Pathology, Laboratory Medicine Region Skåne, Lund, Sweden
  - Department of Clinical Sciences Lund, Division of Oncology, Lund University, Lund, Sweden
- **Bruce Werness, MD**
  - Inova Health System Department of Pathology, Falls Church, VA; bruce.werness@inova.org
  - Arrivebio LLC, San Francisco, CA; bruce.werness@inova.org
- **Amy Ly, MD**
  - Department of Pathology, Massachusetts General Hospital, Boston, MA
- **Xiaoxian Li, MD PhD**
  - Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA
- **Matthew G. Hanna, MD**
  - Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York;
- **Kim RM Blenman, PhD MS**
  - Department of Internal Medicine, Section of Medical Oncology, School of Medicine and Yale Cancer Center, Yale University, New Haven, CT
  - Department of Computer Science, School of Engineering and Applied Sciences, Yale University, New Haven, CT
- **Roberto Salgado, MD PhD**
  - Division of Research, Peter Mac Callum Cancer Centre, Melbourne, Australia
  - Department of Pathology, GZA-ZNA Hospitals, Antwerp, Belgium
- **Brandon D. Gallas, PhD**
  - FDA/CDRH/OSEL/Division of Imaging, Diagnostics, and Software Reliability

# Disclosures

In the past 12 months, I have not had any significant financial interest or other relationship with the manufacturers of the products or providers of the services that will be discussed in my presentation.

The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services. This is a contribution of the U.S. Food and Drug Administration and is not subject to copyright.

# Outline

- High-Throughput Truthing (HTT) Project
- Pilot Study
- Expert Panel Sessions
- New Training Materials
- Upcoming Pivotal Study

# High-Throughput Truthing (HTT) Project

- A collaboration of international volunteers



Pathology  
Innovation  
Collaborative  
Community  
Picc



## eeDAP Studies Group Page

A home for collaborative studies to create tools (methods, data, and code) that advance regulatory science in the area of digital pathology imaging and related artificial intelligence software as a medical device.

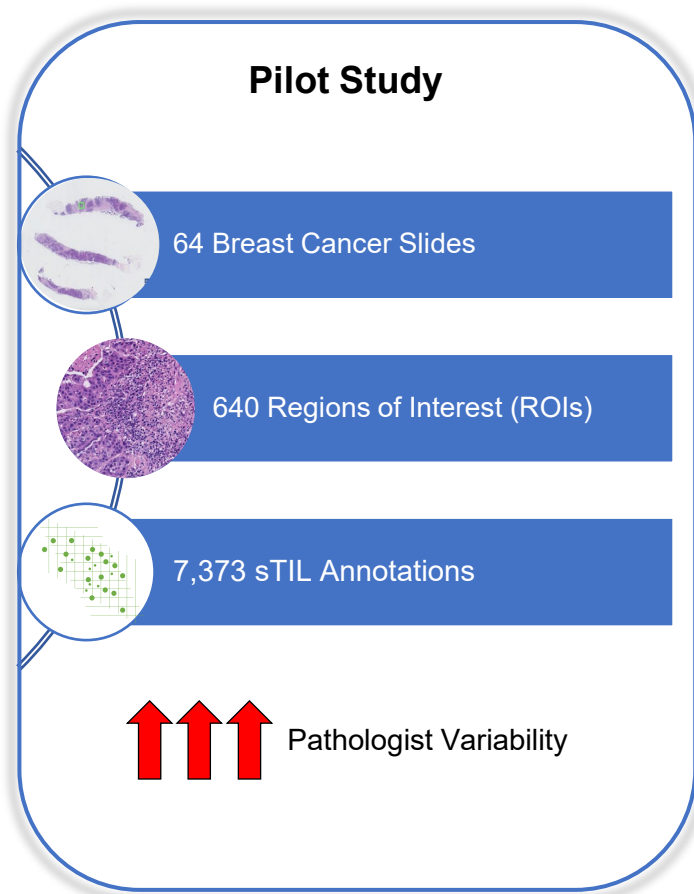
 Wiki Home <small>links to other project pages</small>	 Evaluation Environment for Digital and Analog Pathology (eeDAP)	 Device Advice: for medical device sponsors submitting to the FDA
 What is HTT?	 HTT Data Collection Training	 Start Data Collection

<https://ncihub.org/groups/eedapstudies>

[NCI Hub - Group: eeDAP studies](#) ~ [Wiki: Presentation 2021: Pathology Informatics Summit - HTT project](#)



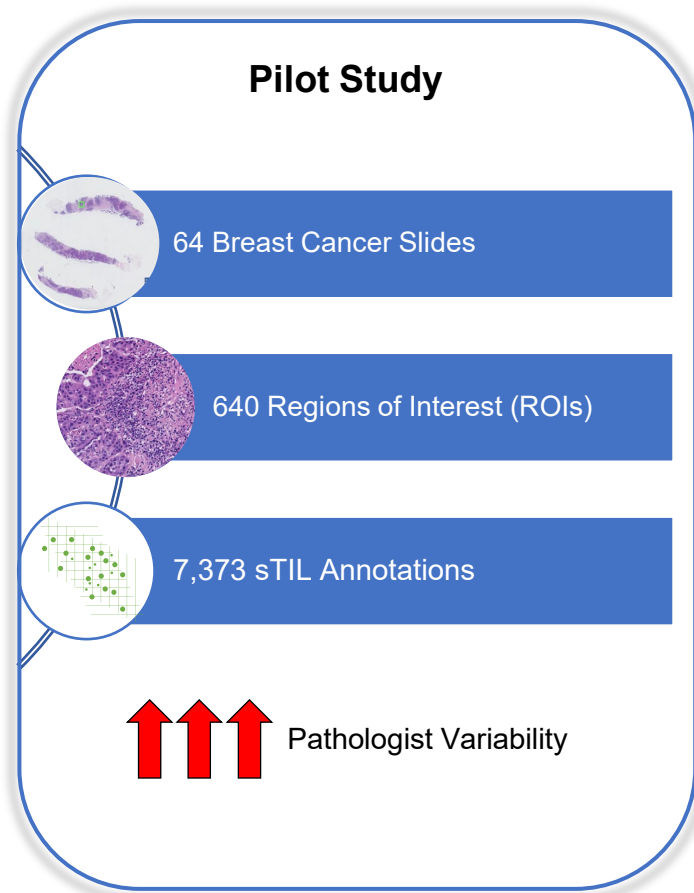
# HTT Pilot Study



Garcia et al., (2022)

- 29 Pathologists
- 2 modalities
  - a light microscope system (eeDAP)
  - two digital whole slide image (WSI) viewing and annotation platforms
    - caMicroscope
    - PathPresenter
- WSI scanned 40x equivalent magnification ( $0.23 \mu\text{m}/\text{pixel}$ )
  - Hamamatsu Nanozoomer 2.0-RS C10730 series
- February 2020 – May 2021

# HTT Pilot Study



Garcia et al., (2022)

- Pilot Study data and Expert Panel annotations are publicly available from our GitHub repository: <https://github.com/DIDSR/HTT>
- Dudgeon SN, Wen S, Hanna MG, et al. A Pathologist-Annotated Dataset for Validating Artificial Intelligence: A Project Description and Pilot Study. *J Pathol Inform.* 2021;12:45. Published 2021 Nov 15. doi:10.4103/jpi.jpi\_83\_20

# caMicroscope Platform

Slide: HTT-TILS-001-29B

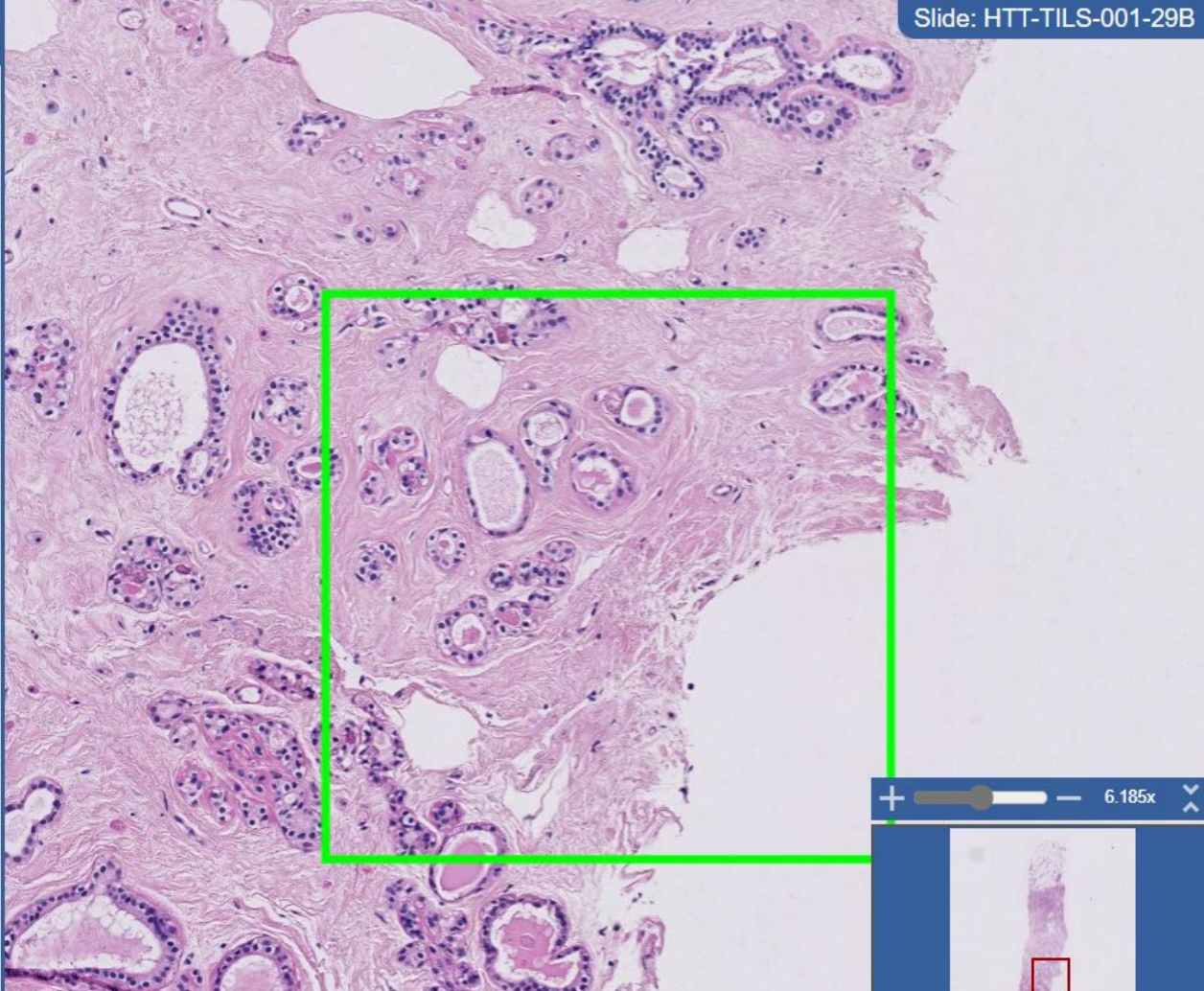
ROI Type:

- Intra-Tumoral Stroma
- Tumor with No Intervening Stroma
- Invasive Margin
- Other Regions

**29%**  
% Tumor-Associated Stroma

**71%**  
sTIL Density

Save & Next



The image shows a histological section of tissue stained with hematoxylin and eosin (H&E). A green rectangular region of interest (ROI) is drawn over a portion of the tissue, highlighting glandular structures and stroma. A zoomed-in inset of this ROI is shown in the bottom right corner, with a red box indicating the area being magnified. The zoom level is set to 6.185x. The interface includes navigation icons at the top left and a 'Save & Next' button at the bottom of the control panel.



# Collected Annotations

**ROI Type:**

- Intra-Tumoral Stroma
- Tumor with No Intervening Stroma
- Invasive Margin
- Other Regions

**48%**  
% Tumor-Associated Stroma

**84%**  
Please Assess TIL Density

Save & Next

- Region of Interest (ROI) Label
  - Describes the tissue within the ROI
- % Tumor-Associated Stroma
  - The percentage of tumor-associated stroma present within the ROI

$$\left( \frac{\text{Area of Tumor-Associated Stroma}}{\text{Area of Entire ROI}} \right) \times 100\%$$

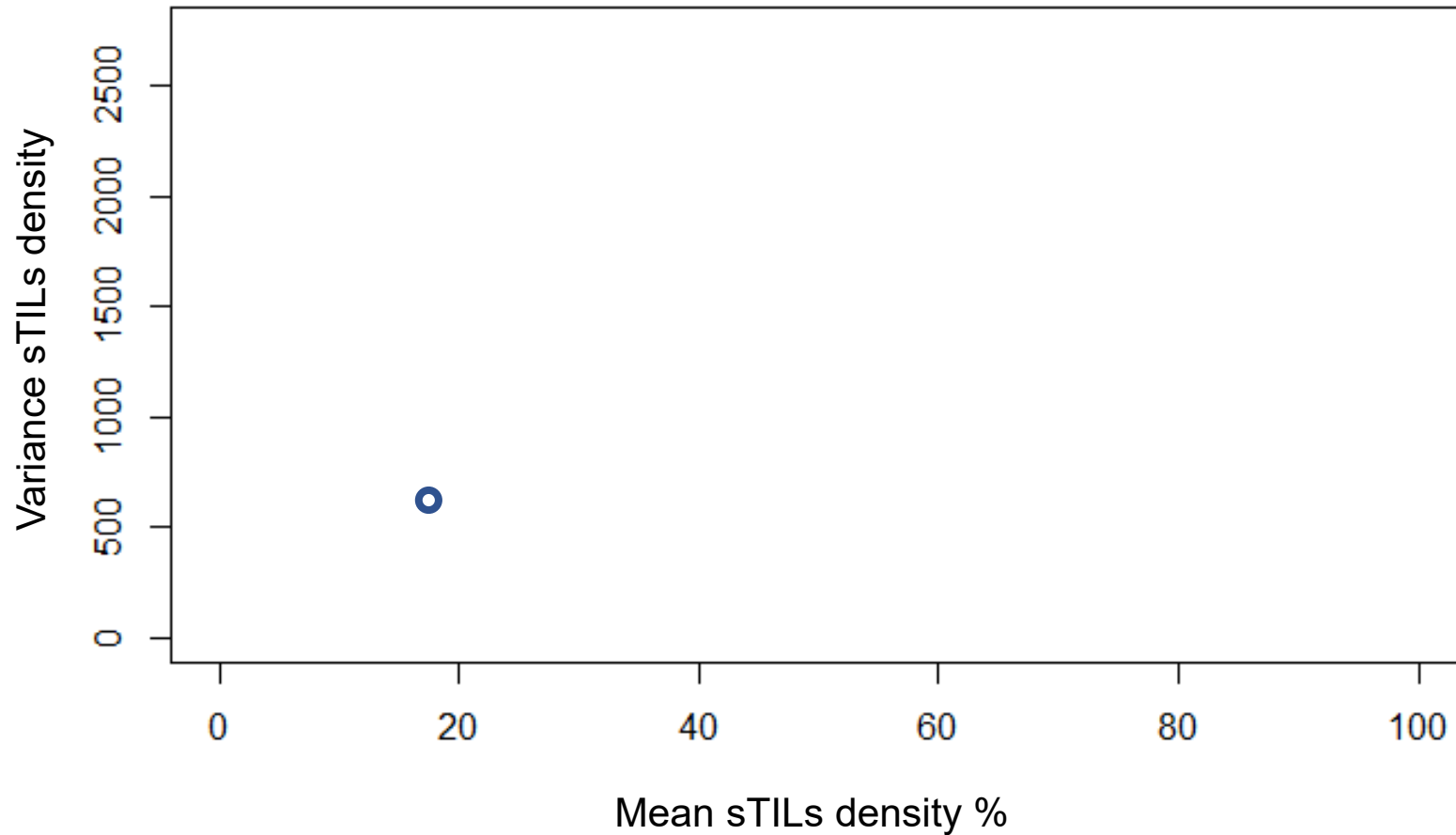
- sTILs Density
  - The percentage of TILs area within tumor-associated stroma

$$\left( \frac{\text{Area of Tumor-Infiltrating Lymphocytes}}{\text{Area of Tumor-Associated Stroma}} \right) \times 100\%$$

Garcia et al., (2022)

# Initial Analysis of Pilot Study

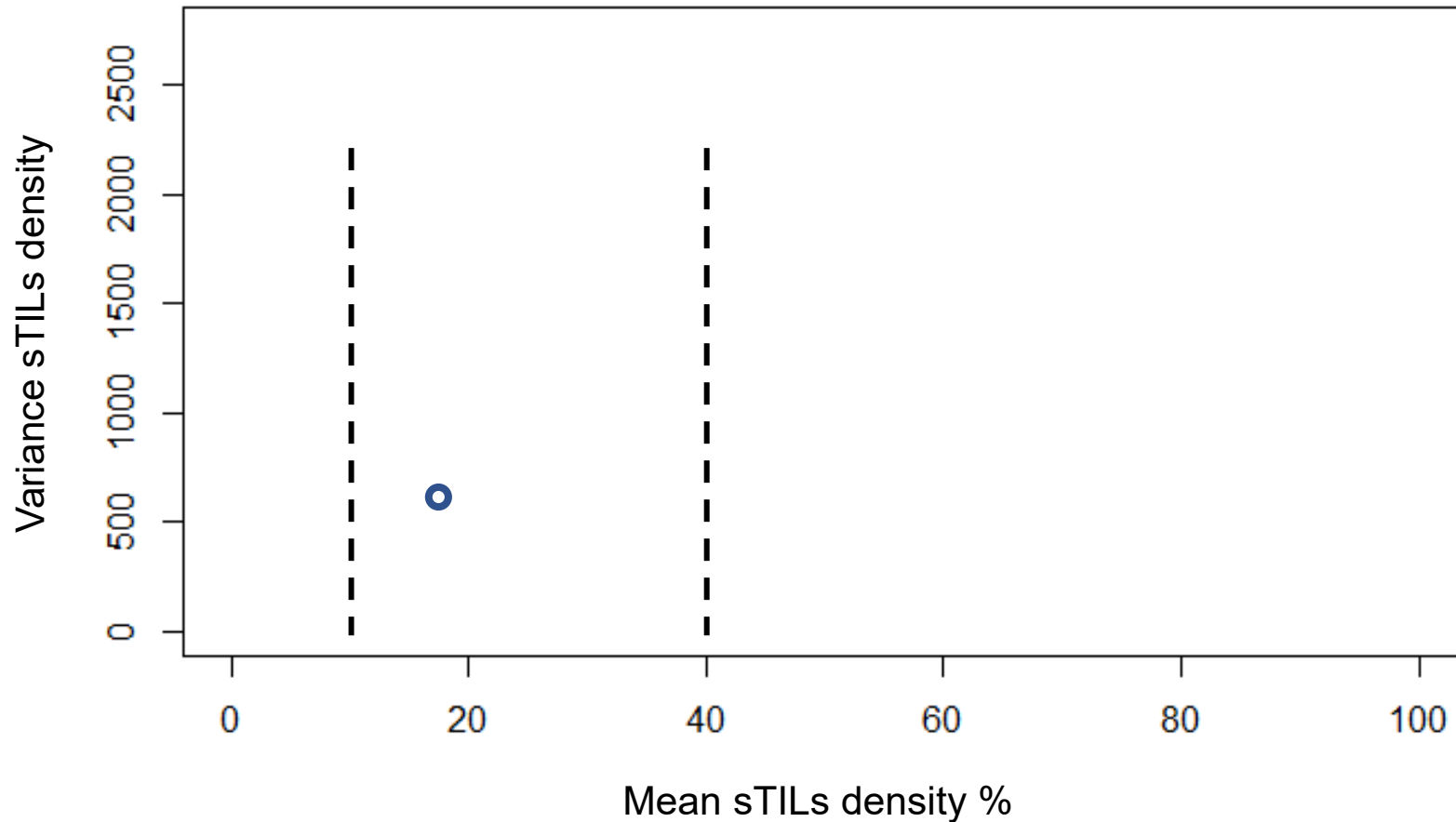
**Variance of Pilot Study**



- Mean and Variance are averages over all readers

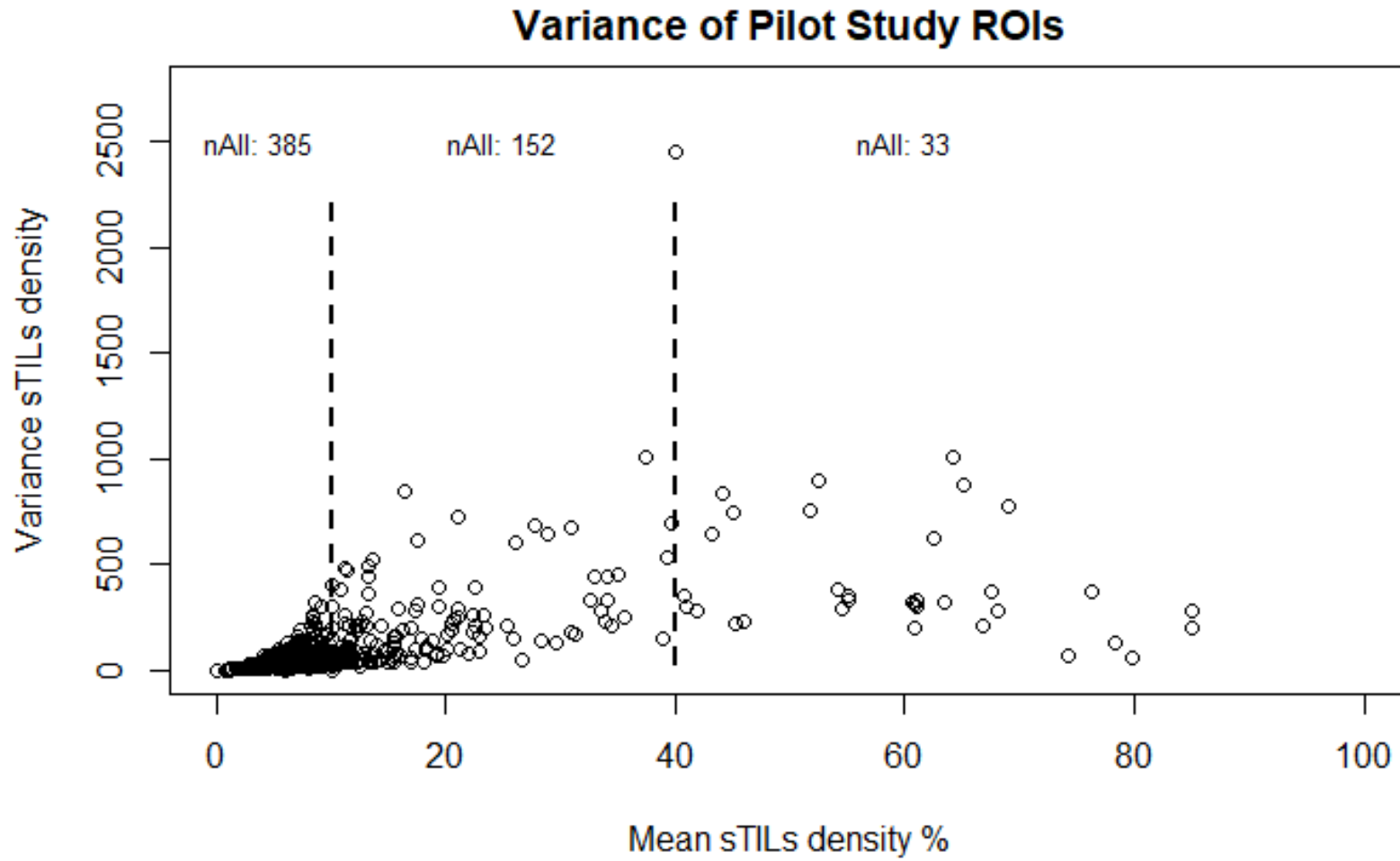
# Initial Analysis of Pilot Study

**Variance of Pilot Study**



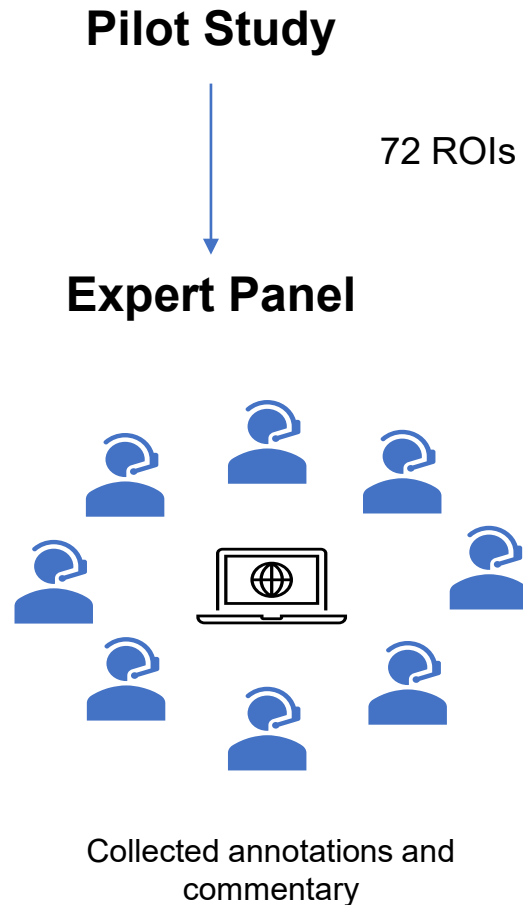
- Mean and Variance are averages over all readers
- Vertical dashed lines represent clinical bins
  - low ( $\leq 10\%$ )
  - medium ( $>10\% \ \& \ \leq 40\%$ )
  - high ( $>40\%$ )

# Initial Analysis of Pilot Study



- Mean and Variance are averages over all readers
- Vertical dashed lines represent clinical bins
  - low ( $\leq 10\%$ )
  - medium ( $>10\% \ \& \ \leq 40\%$ )
  - high ( $>40\%$ )
- To reduce study Variance, we used an Expert Panel to create new Training Materials

# Expert Panel Sessions



- 72 “Select” ROIs
- 8-member expert panel
- 8 recorded, one-hour virtual sessions
- Collect annotations independently
  - Digital mode: caMicroscope

Garcia et al., (2022)

# ROI Selection: Variance and Entropy

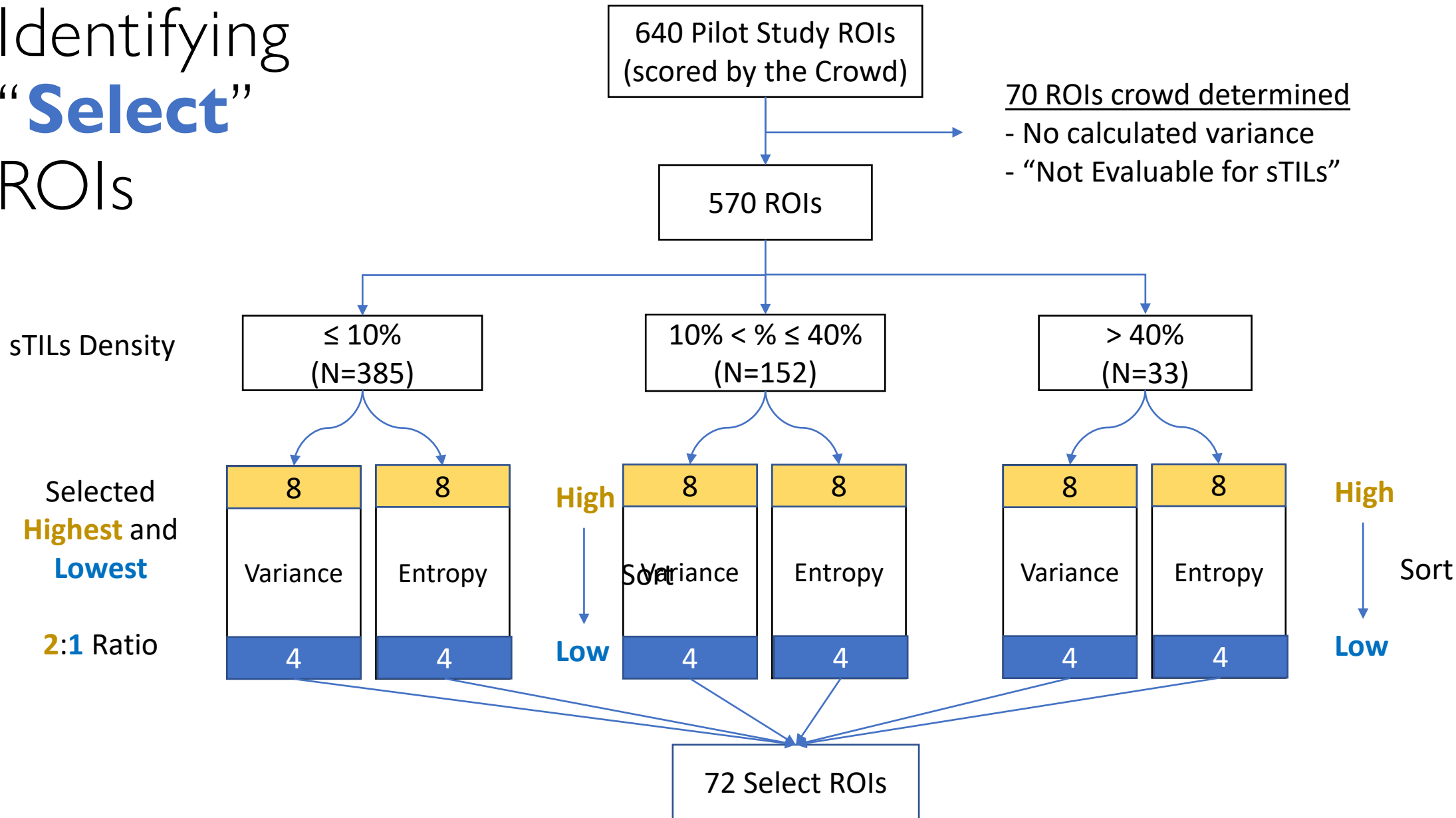
- Variance
  - Variance over pathologist's sTILs Density estimates
- Entropy
  - Measure of variance for categorical data (ROI Type)
  - Captures the number of different ROI Types and the frequency of the chosen ROI Types.

$$\text{Entropy} = -\sum_{i=1}^4 p_i \log(p_i)$$

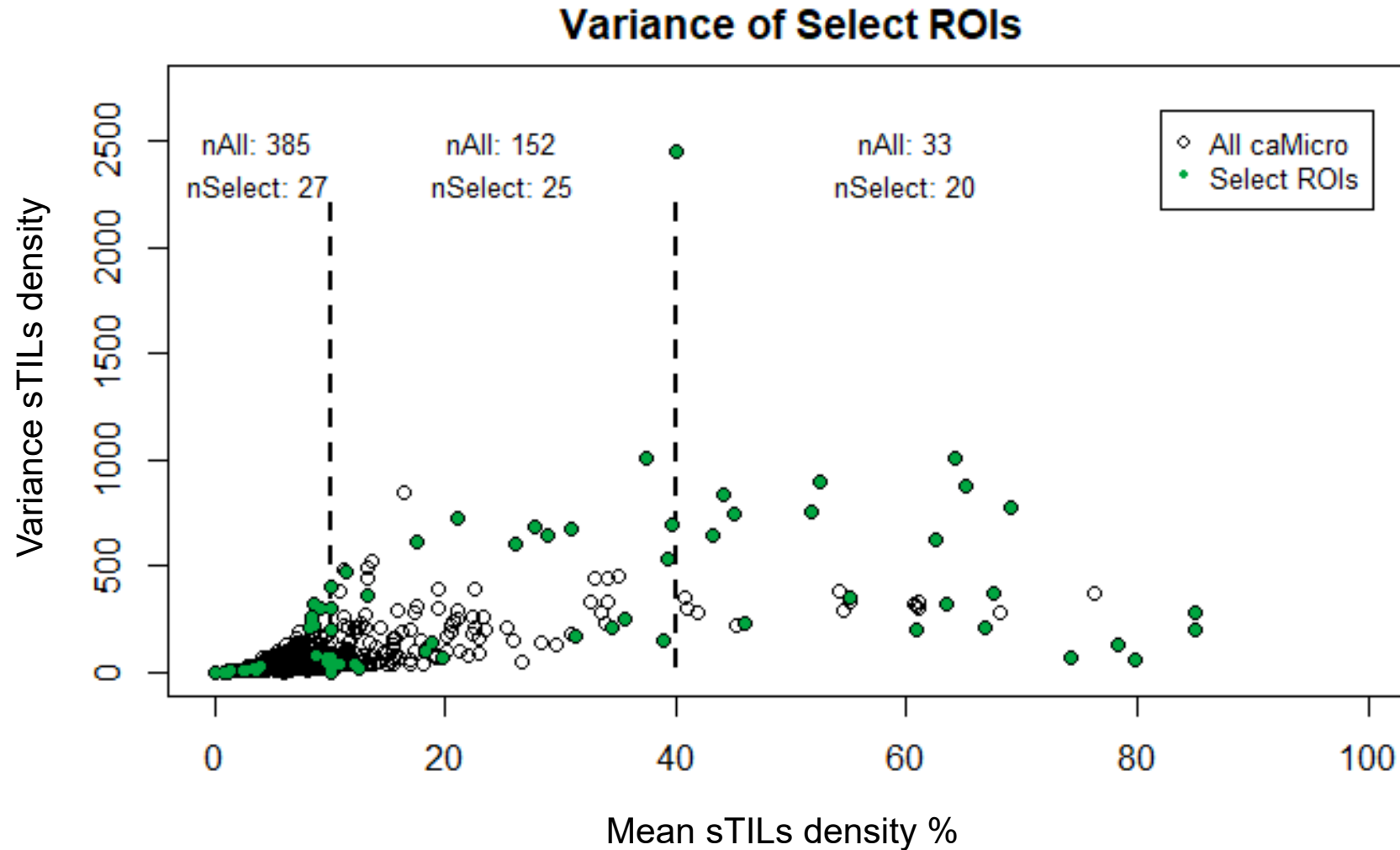
## ROI Type:

- Intra-Tumoral Stroma
- Tumor with No Intervening Stroma
- Invasive Margin
- Other Regions

# Identifying “**Select**” ROIs



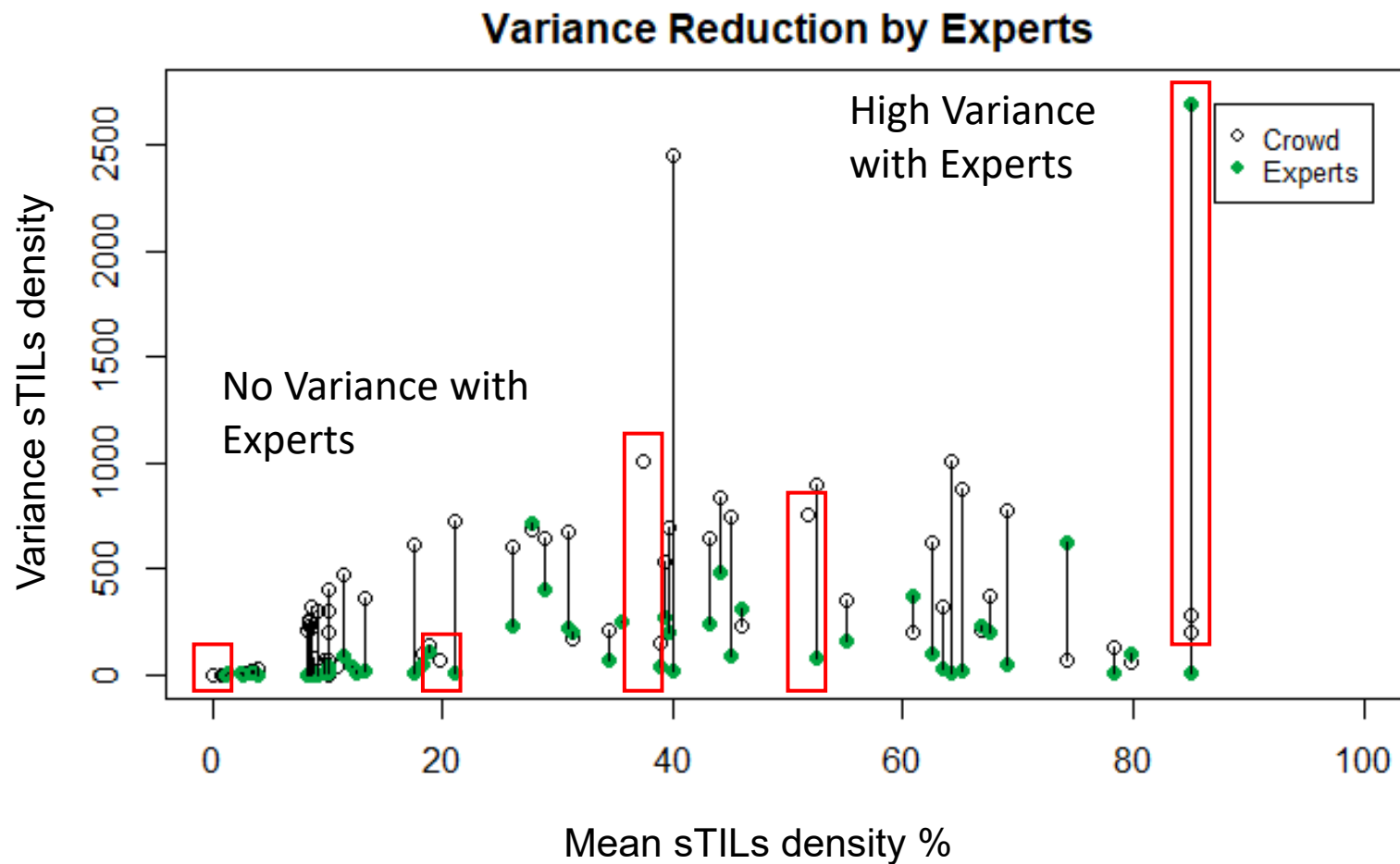
# Variance



Garcia et al., (2022)



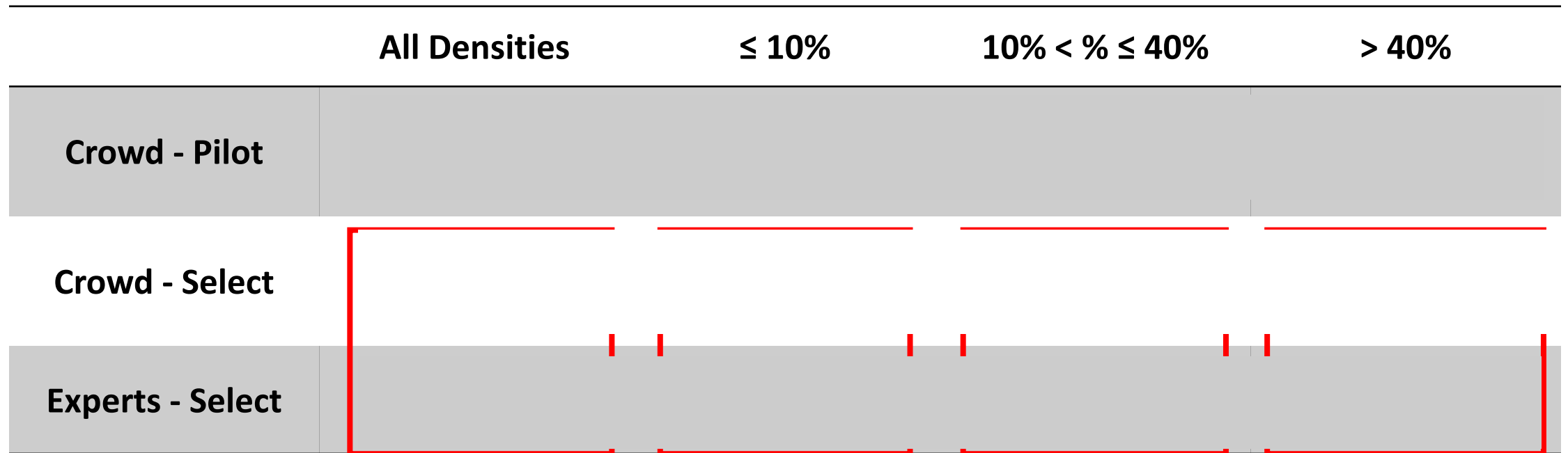
# Expert Panel Annotations: Reduction in Variance



Garcia et al., (2022)

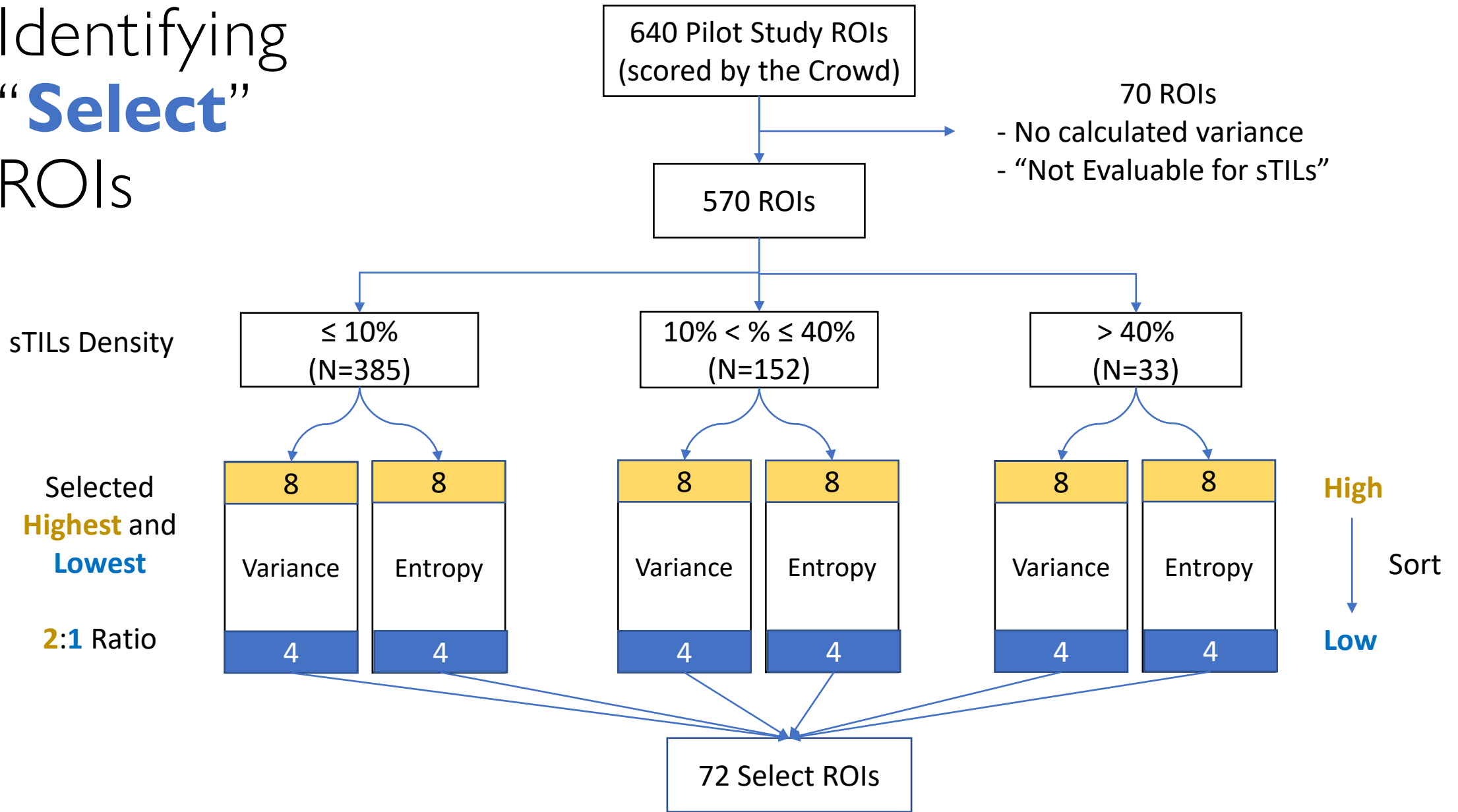
# Expert Panel Annotations: Reduction in Variance

Legend:  
Median [IQR]

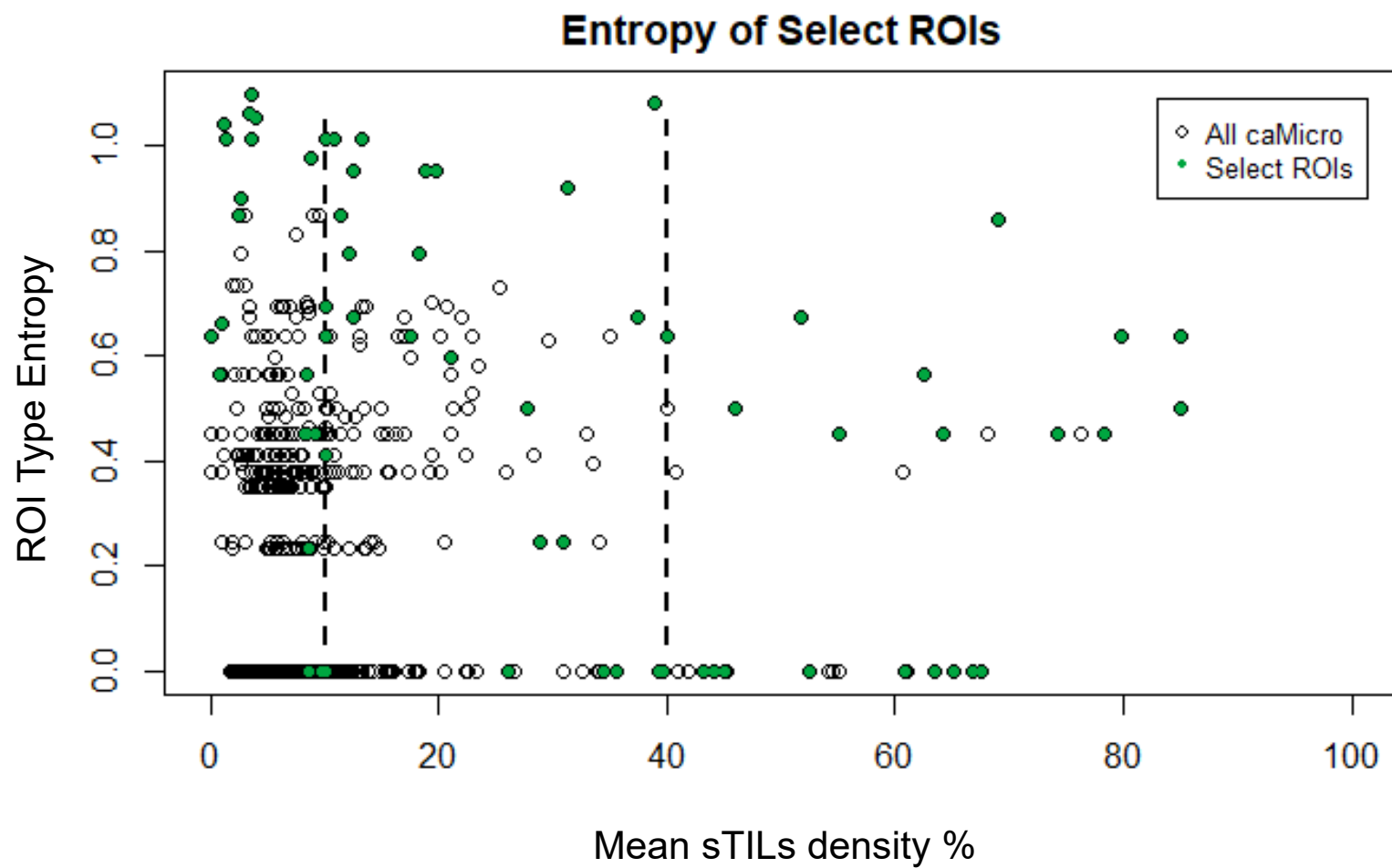


Garcia et al., (2022)

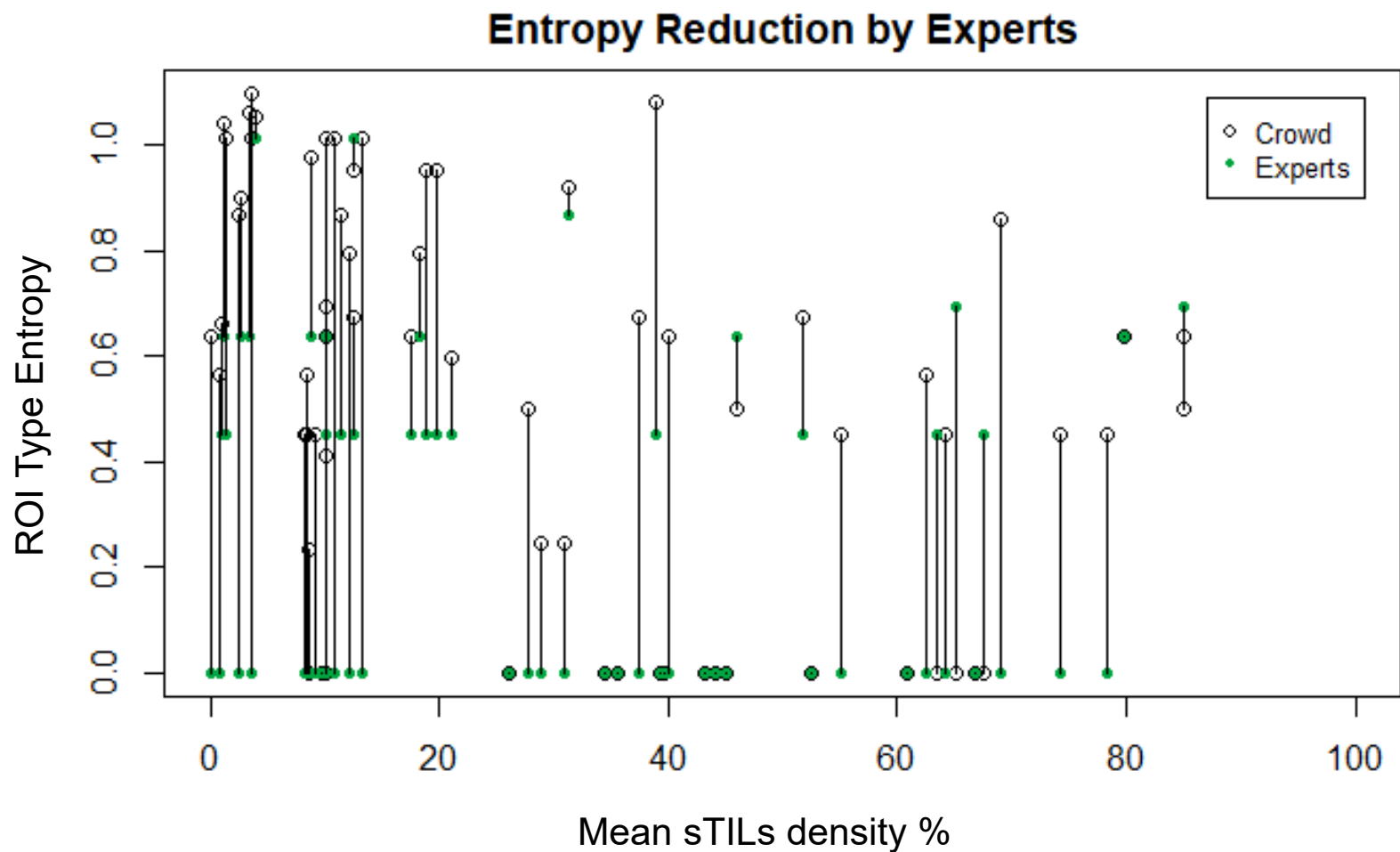
# Identifying “**Select**” ROIs



# Entropy



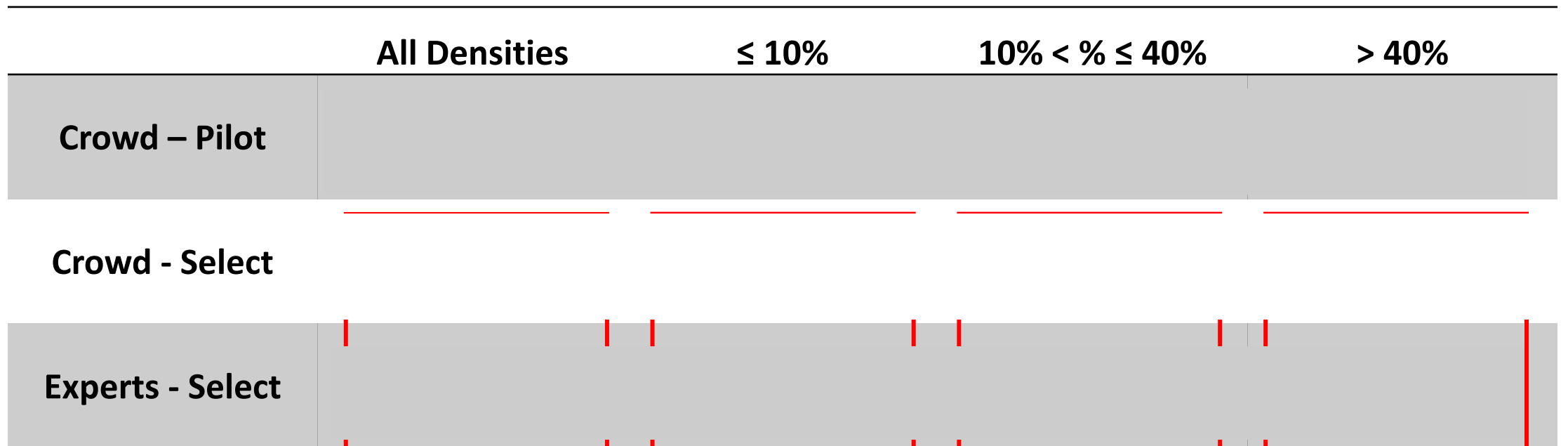
# Expert Panel Annotations: Reduction in Entropy



Garcia et al., (2022)

# Expert Panel Annotations: Reduction in Entropy

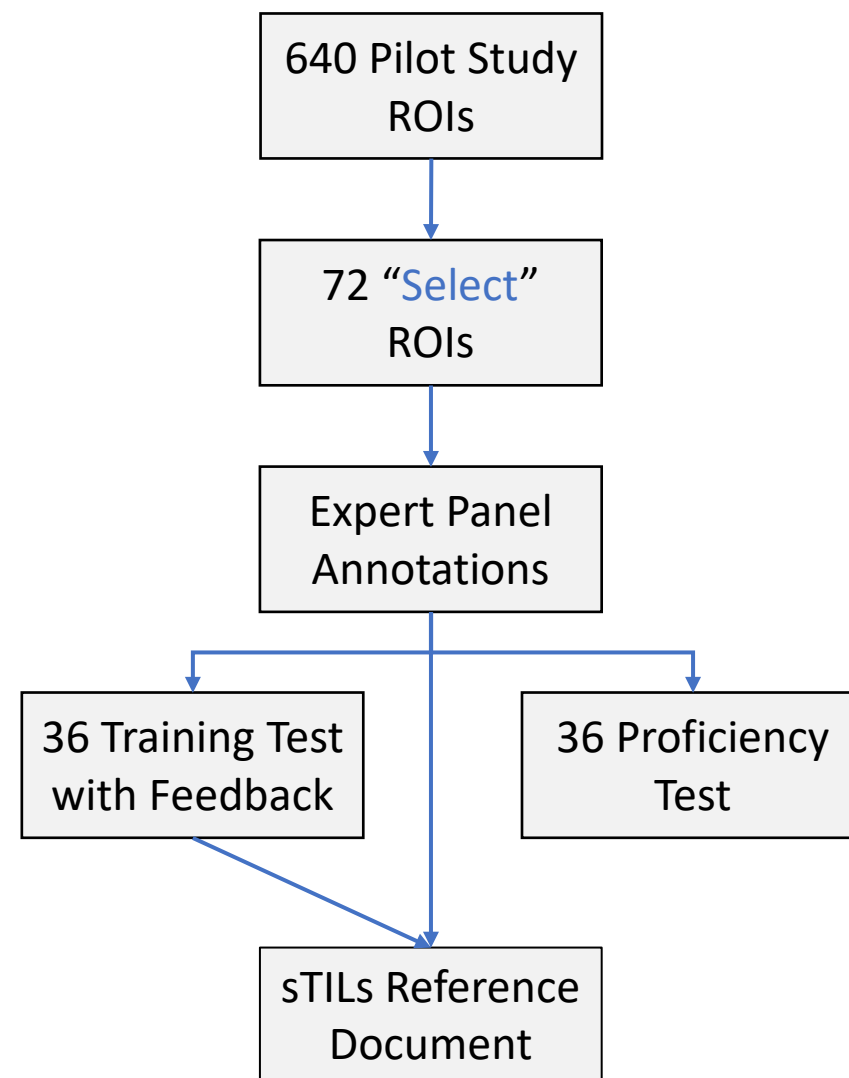
Legend:  
Median [IQR]



Garcia et al., (2022)

# New Training Materials

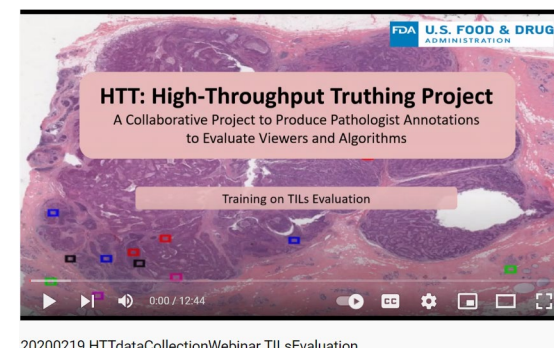
- sTILs Reference Document
- Training Test with Feedback
- Proficiency Test



# Pilot Study Training Materials

- Recorded webinar from 2020 USCAP Annual Meeting data collection event
  - [20200219 HTTdataCollectionWebinar TILsEvaluation – YouTube](#)

reviews



*Annals of Oncology* 26: 259–271, 2015  
doi:10.1093/annonc/mdu450  
Published online 11 September 2014

## The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014

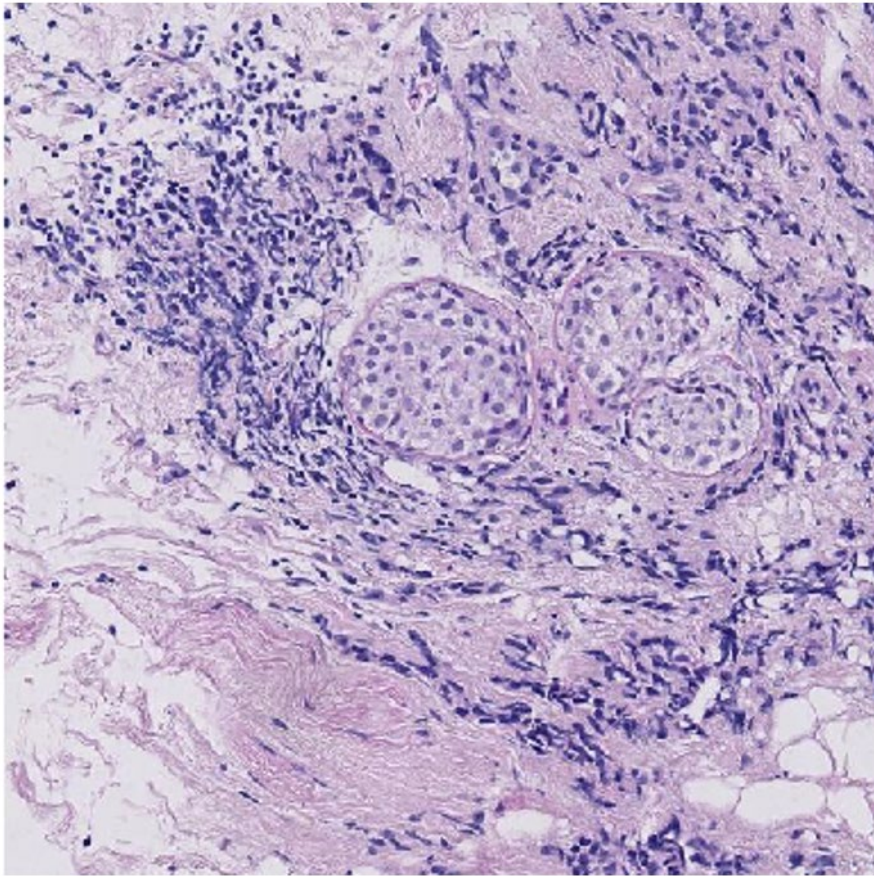
R. Salgado<sup>1,2,†</sup>, C. Denkert<sup>3,†</sup>, S. Demaria<sup>4,†</sup>, N. Sirtaine<sup>5</sup>, F. Klauschen<sup>3</sup>, G. Pruneri<sup>6</sup>, S. Wienert<sup>3</sup>, G. Van den Eynden<sup>7</sup>, F. L. Baehner<sup>8,9</sup>, F. Penault-Llorca<sup>10</sup>, E. A. Perez<sup>11</sup>, E. A. Thompson<sup>12</sup>, W. F. Symmans<sup>13</sup>, A. L. Richardson<sup>14,15</sup>, J. Brock<sup>15,16</sup>, C. Criscitiello<sup>17</sup>, H. Bailey<sup>8</sup>, M. Ignatiadis<sup>18</sup>, G. Floris<sup>19</sup>, J. Sparano<sup>20</sup>, Z. Kos<sup>21</sup>, T. Nielsen<sup>22</sup>, D. L. Rimm<sup>23</sup>, K. H. Allison<sup>24</sup>, J. S. Reis-Filho<sup>25</sup>, S. Loibl<sup>26</sup>, C. Sotiriou<sup>18</sup>, G. Viale<sup>27</sup>, S. Badve<sup>28</sup>, S. Adams<sup>4,†</sup>, K. Willard-Gallo<sup>29,†</sup> & S. Loi<sup>30\*,†</sup>



# sTILs Reference Document

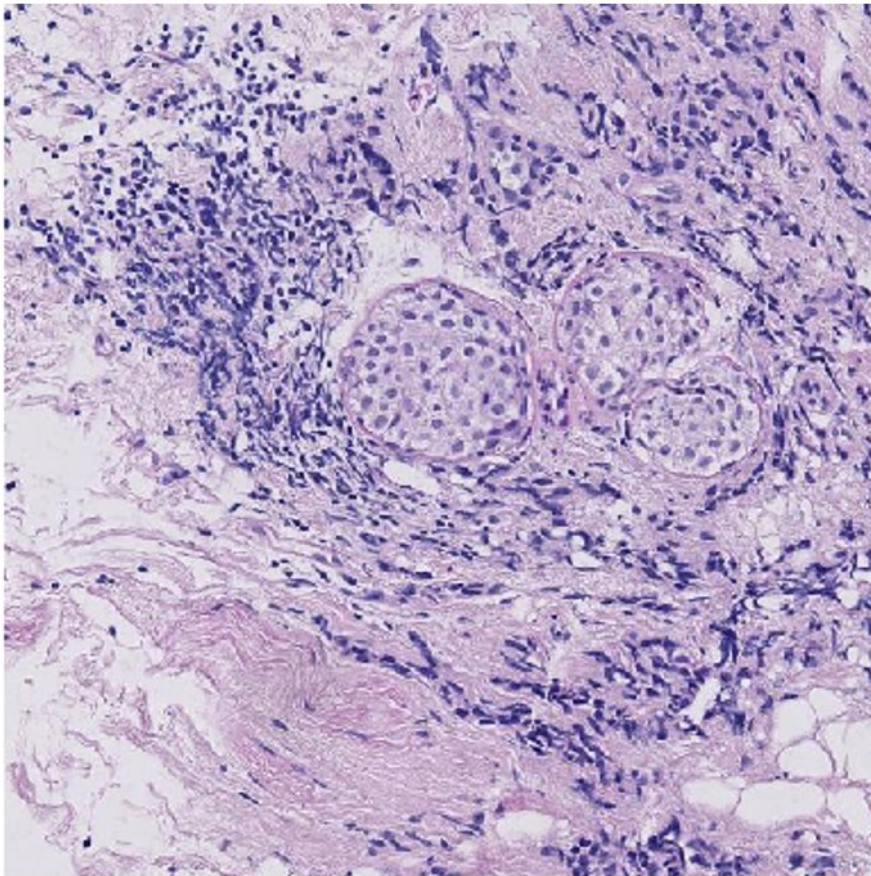
- Collective of experts' annotations, comments, and pitfalls
- Introduction
- Performing the sTILs Assessment
- Pitfalls in sTILs Assessment
- Clinical Pearls for Approaching sTILs Assessment
- Annotations and Discussions

# sTILs Reference Document



caseID: HTT-TILS-001-27B.ndpi\_x5114.2190\_y25709.2190

# sTILs Reference Document



**Comments:** A challenging ROI. The DCIS in the center of the ROI is excluded from the calculation. Crushed tumor cells may be confused for lymphocytes. There are few small foci of invasive tumor (e.g. in upper third of ROI) that would be the focus of assessment. The lower part of ROI is also not reliable for scoring due to marked crush artifact. Crushed cells in the lower right quarter are suspicious for carcinoma, and not TILs, based on architecture similar to tumor cells outside of this ROI.

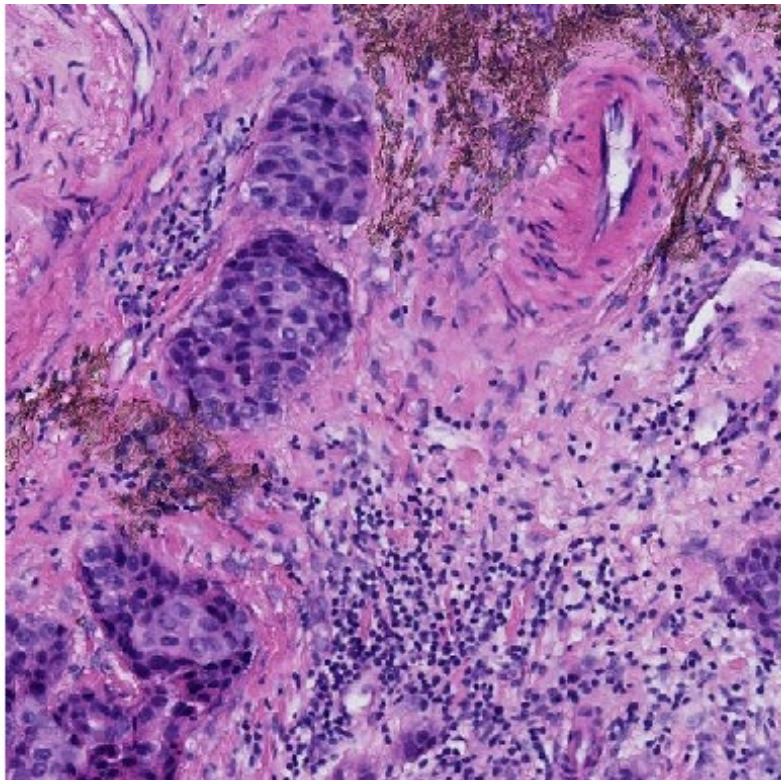
**Pitfalls:** DCIS is excluded from the numerator when calculating the percentage of tumor-associated stroma. Non-lymphocytes may be confused for lymphocytes if there is tissue fixation/preservation artifacts. Comparison with areas outside of the ROI may help overcome tissue/fixation artifacts.

caseID: HTT-TILS-001-27B.ndpi\_x5114.2190\_y25709.2190

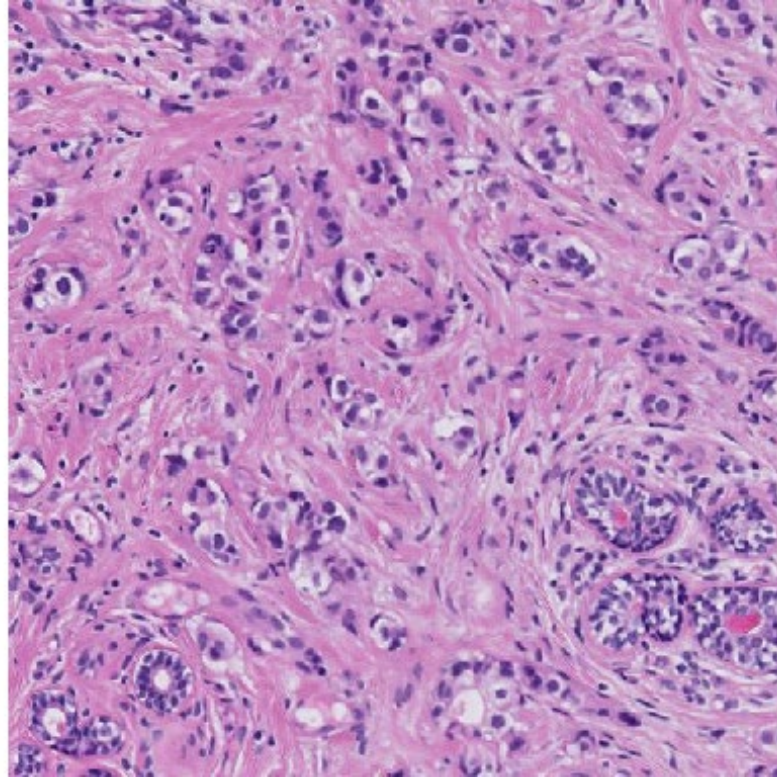
# Pitfalls in Percent Tumor-Associated Stroma

- Exclude thick-walled vessels, benign glandular elements, adipocytes, carcinoma in situ, and necrosis from the area of tumor-associated stroma
- Calculate with respect to the entire ROI area
- Variations in tumor cell morphology can make it difficult to distinguish stroma from tumor

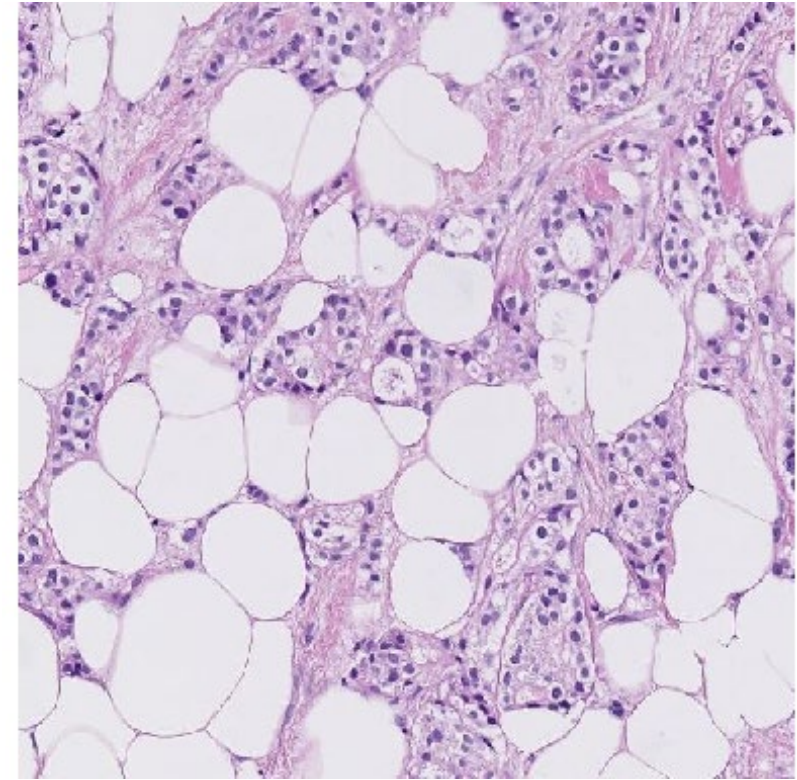
Exclude thick-walled vessels, benign glandular elements, adipocytes, carcinoma in situ, and necrosis from the area of tumor-associated stroma



Thick-walled vessels



Benign glandular elements

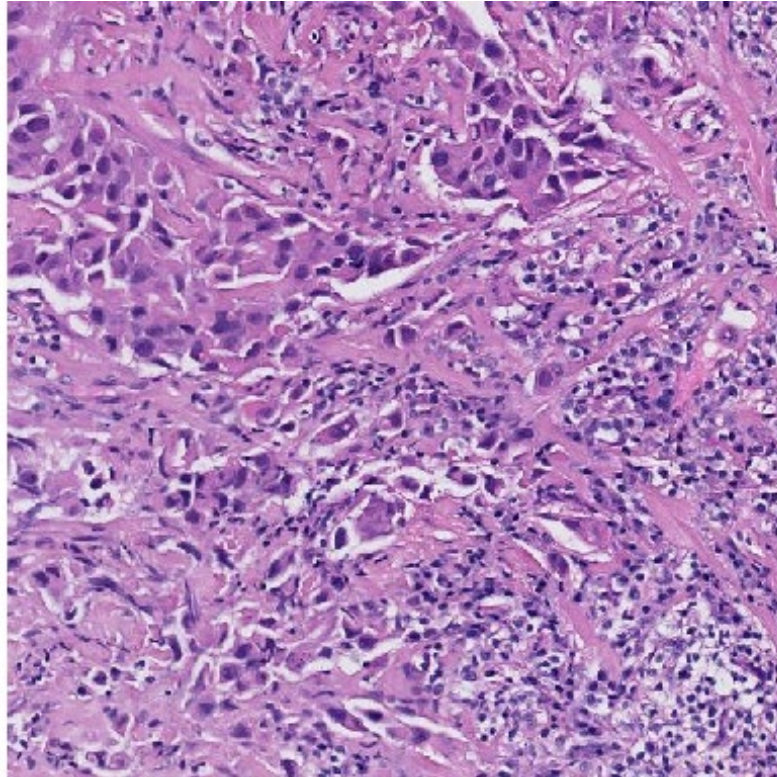


Adipocytes

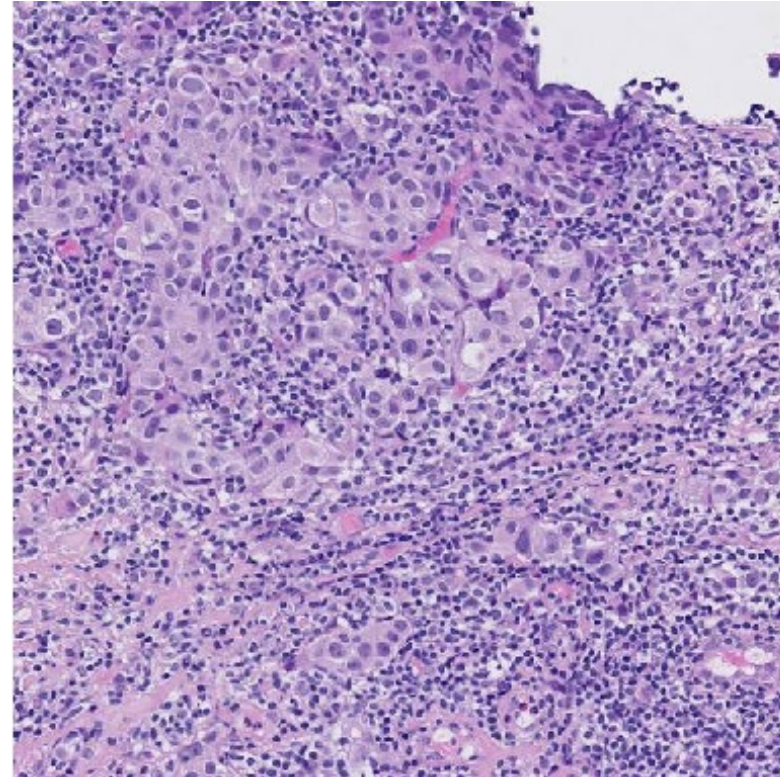
# Pitfalls in sTILs Density

- Cells with small/pyknotic nuclei and/or perinuclear clearing can be difficult to categorize
- Non-lymphoid cells may be confused for lymphocytes
- Error in the percent tumor-associated stroma can affect the sTILs density
- Sparsely distributed tumor cells may be more challenging to quantitate

Cells with small/pyknotic nuclei and/or perinuclear clearing can be difficult to categorize



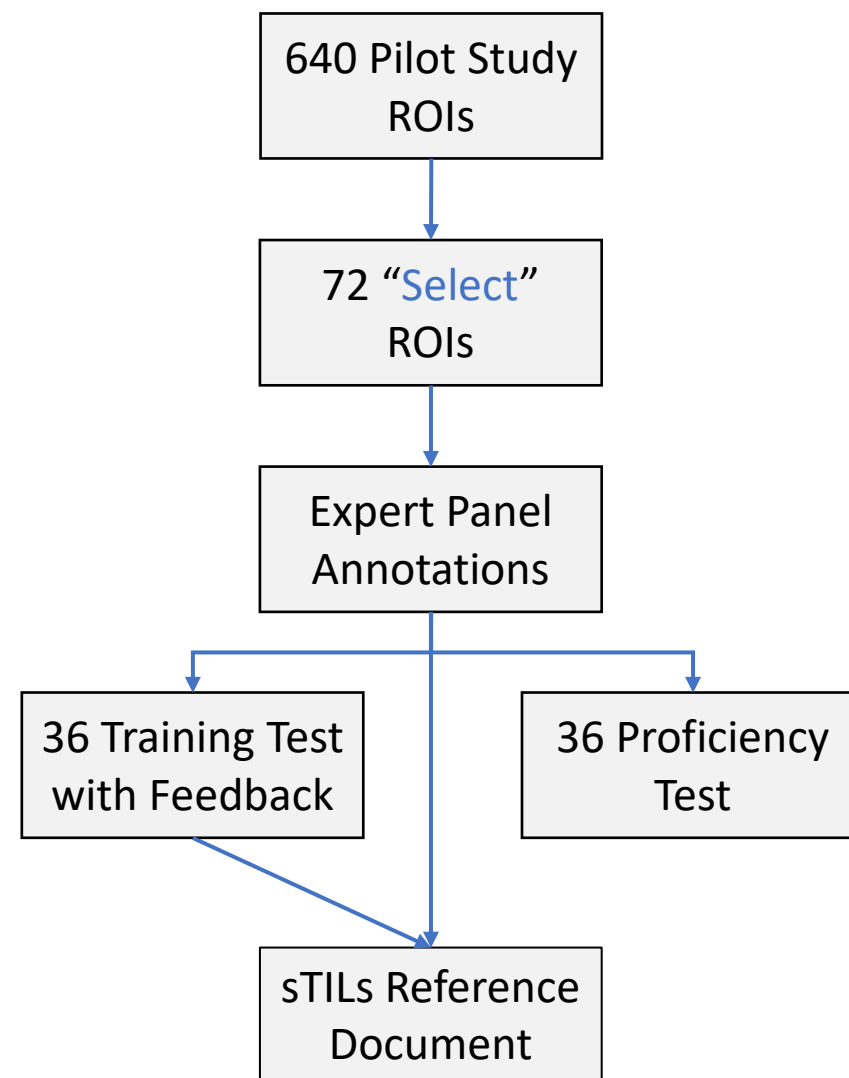
Perinuclear clearing



Pyknotic tumor cells

# New Training Materials

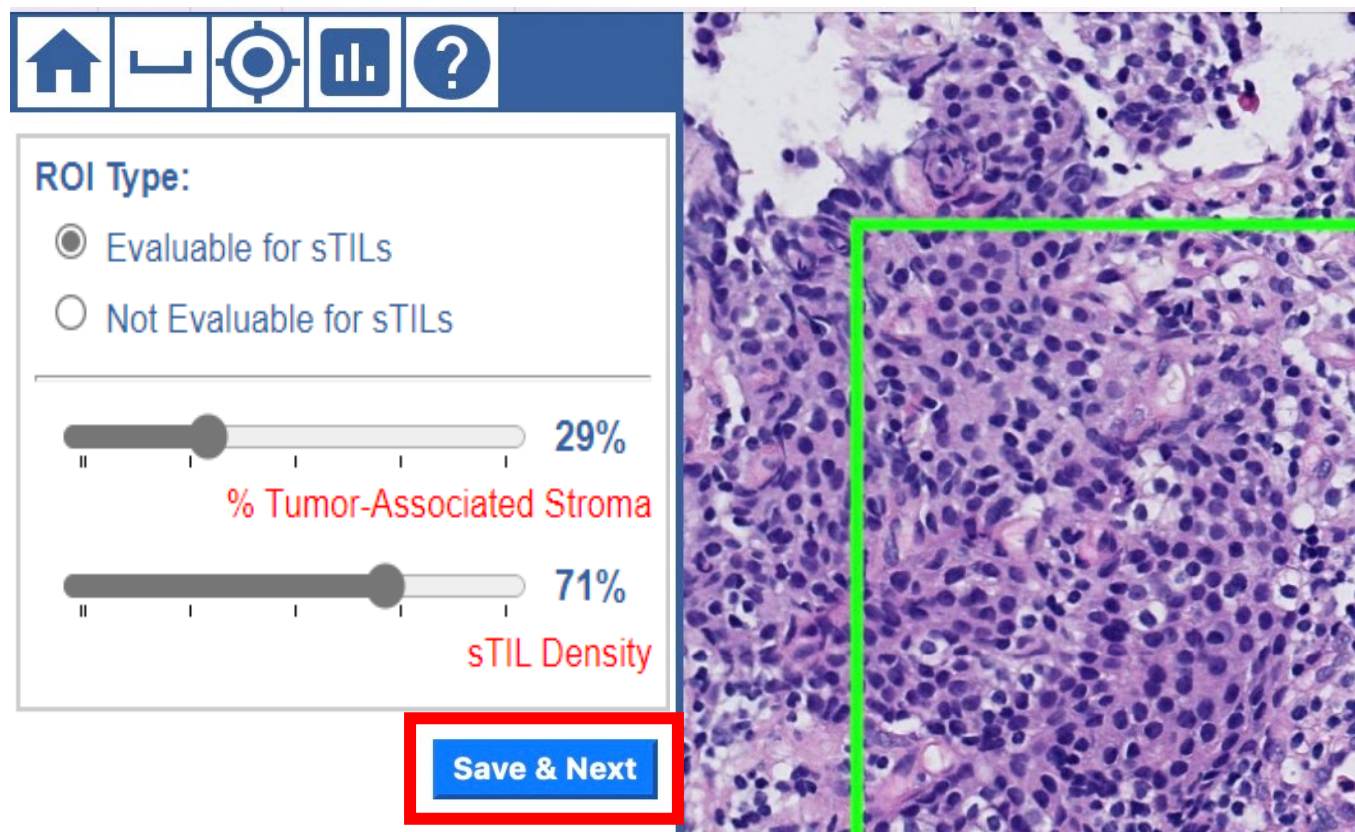
- sTILs Reference Document
- Training Test with Feedback
- Proficiency Test





# Training Test with Feedback

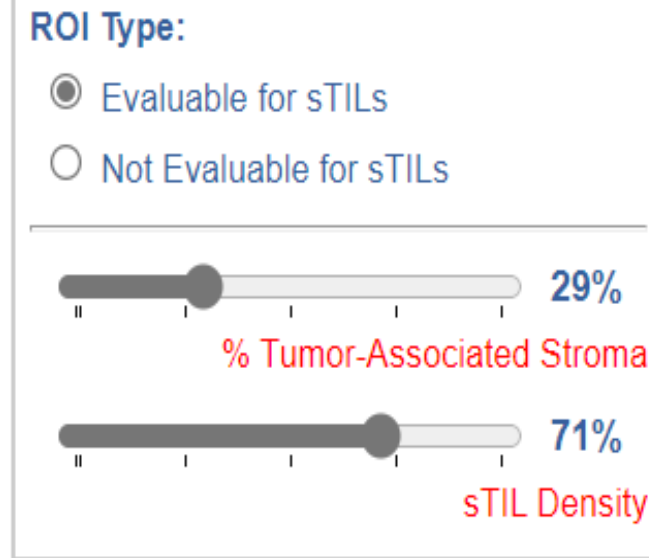
- 36 ROIs from Expert Panel
- “Evaluable for sTILs” or “Evaluable”
  - The ROI should be considered for the sTILs assessment



The screenshot displays a software interface for evaluating a Region of Interest (ROI) in a histology image. The interface includes a navigation bar with icons for home, back, settings, data, and help. Below the navigation bar, there is a section for "ROI Type" with two radio button options: "Evaluable for sTILs" (selected) and "Not Evaluable for sTILs". Two sliders are visible: the first is labeled "% Tumor-Associated Stroma" and is set to 29%; the second is labeled "sTIL Density" and is set to 71%. A blue button labeled "Save & Next" is highlighted with a red border. The background image is a histology slide with a green rectangular ROI box overlaid on a dense area of cells.

# Training Test with Feedback

- 36 ROIs from Expert Panel
- “Evaluable for sTILs” or “Evaluable”
  - The ROI should be considered for the sTILs assessment



## Expert Panel Annotations:

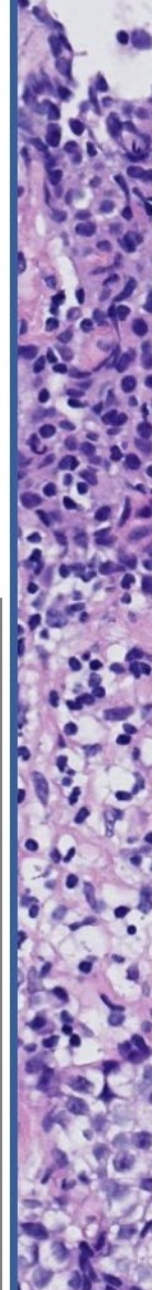
ROI Type	% Tumor-Associated Stroma	% sTIL Density
Evaluable	75	30
Evaluable	35	60
Evaluable	86	15
Evaluable	75	30
Evaluable	70	25
Evaluable	70	20

Mean Percent Stroma: 68.5

Mean sTIL Density: 30

Comments: NA

**Pitfalls:** The stromal percentage is calculated with respect to the area of the entire ROI. Negative/empty space is to be included in the total ROI area, the denominator or percent stroma.



# Proficiency Test

- 36 ROIs from Expert Panel
  - Separate from Training Test with Feedback
- Assess ROIs without any feedback
- sTILs assessment performance compared against Expert's annotations
- Participants who perform above a specified metric can complete the pivotal study

# Updates to data collection tools / methods

- Made improvements to digital platforms
  - Required registration
  - Workflow updates
- New hardware for faster operation of eeDAP
- Updated ROI Type data element for Pivotal Study
  - “Evaluable for sTILs” and “Not evaluable for sTILs”
- ROI images (Github) and full WSI (caMicroscope) can now be downloaded
  - Soon to have bulk WSI download option

# Upcoming Pivotal Study

- Actively sourcing slides
- Working on an application for a CME course
- Curate and prepare data
- Pivotal study launch this summer
  - Looking for expert pathologists

# Get Involved!

- Want to learn more?
  - Visit our eeDAP NCI Hub public page <https://ncihub.org/groups/eedapstudies>
- Want to collaborate?
  - Join our team in Plcc [Truthing & Validations — Plcc Alliance \(pathologyinnovationcc.org\)](https://pathologyinnovationcc.org)
  - Slide sourcing for our pivotal study
- Contact
  - Brandon D. Gallas, PhD
    - [Brandon.gallas@fda.hhs.gov](mailto:Brandon.gallas@fda.hhs.gov)
  - Victor Garcia, MD
    - [Victor.Garcia@fda.hhs.gov](mailto:Victor.Garcia@fda.hhs.gov)

# QUESTIONS?

**Pathology  
Informatics  
Summit 2022**

David L. Lawrence Convention Center  
The Westin Pittsburgh

# Additional ROI Examples

Pitfalls in Percent Tumor-Associated Stroma

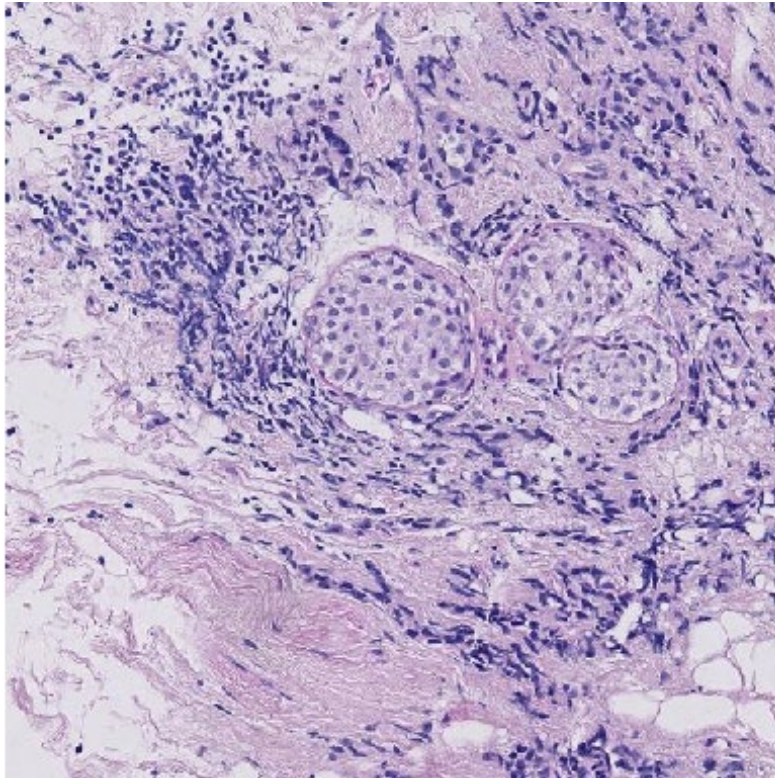
**Pathology  
Informatics  
Summit 2022**

David L. Lawrence Convention Center  
The Westin Pittsburgh

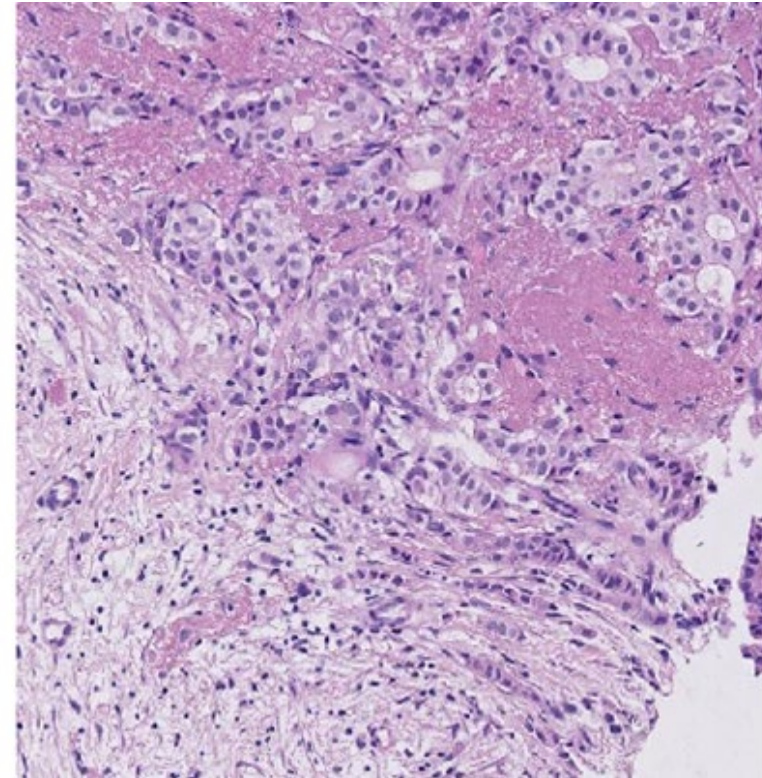




Exclude thick-walled vessels, benign glandular elements, adipocytes, carcinoma in situ, and necrosis from the area of tumor-associated stroma

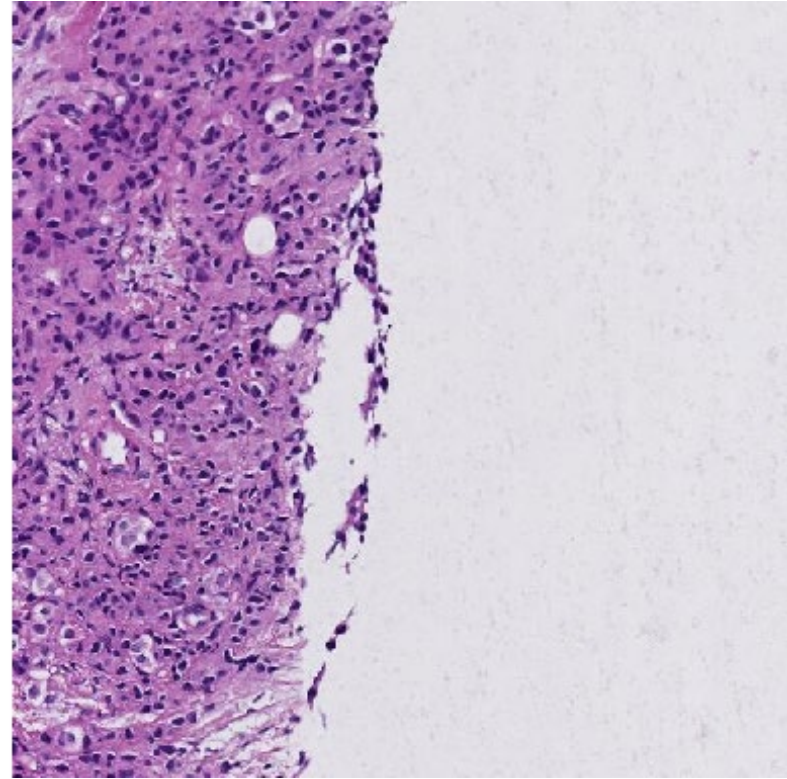
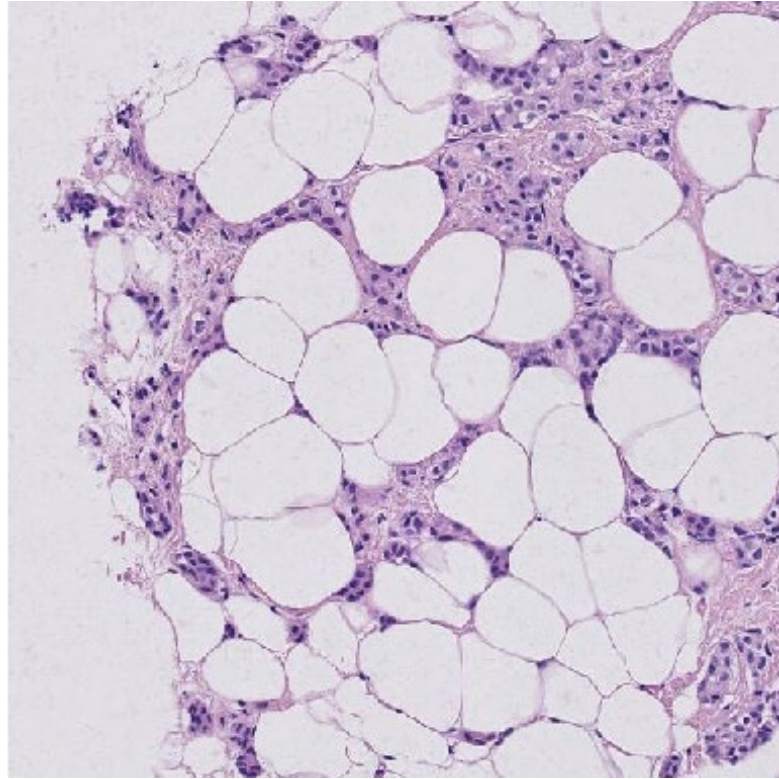


Carcinoma in situ

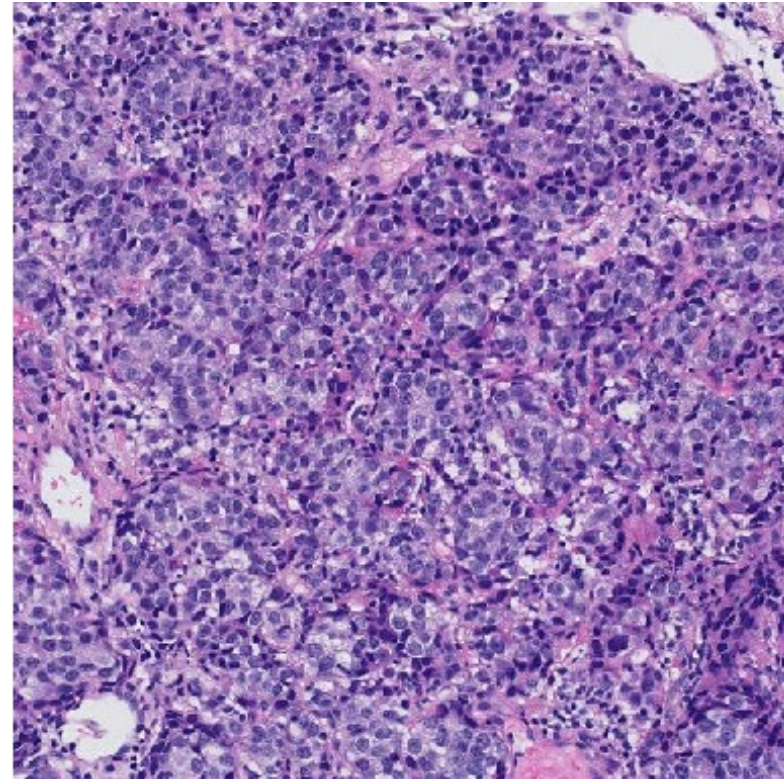
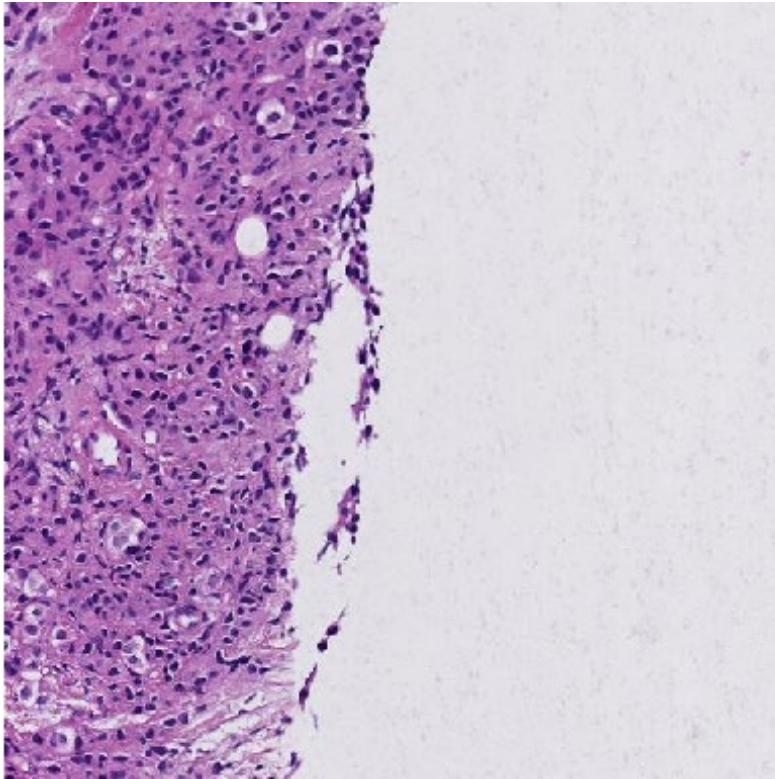


Necrosis

# Calculate with respect to the entire ROI area



Variations in tumor cell morphology can make it difficult to distinguish stroma from tumor



# Additional ROI Examples

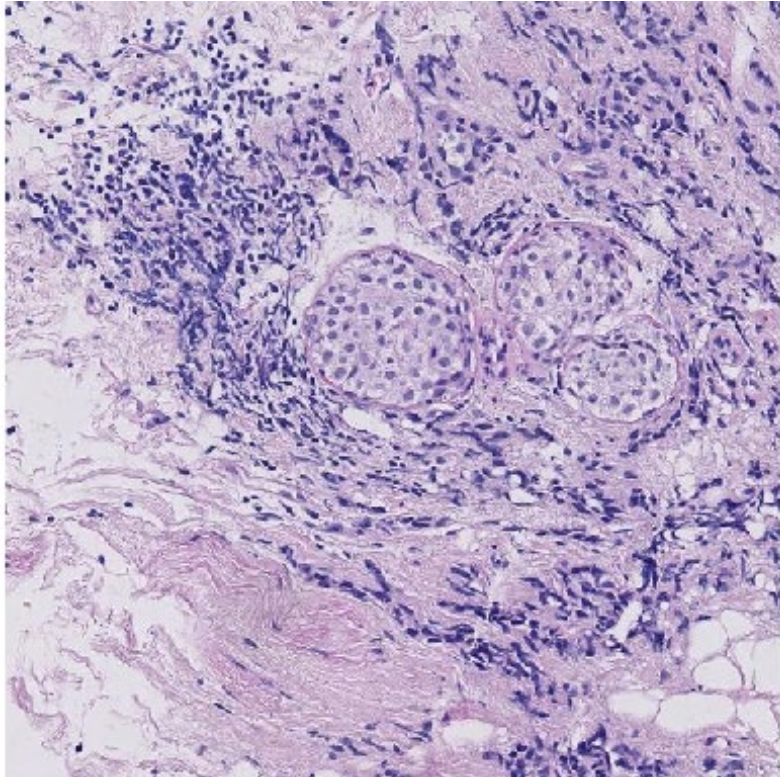
Pitfalls in sTILs Density

**Pathology  
Informatics  
Summit 2022**

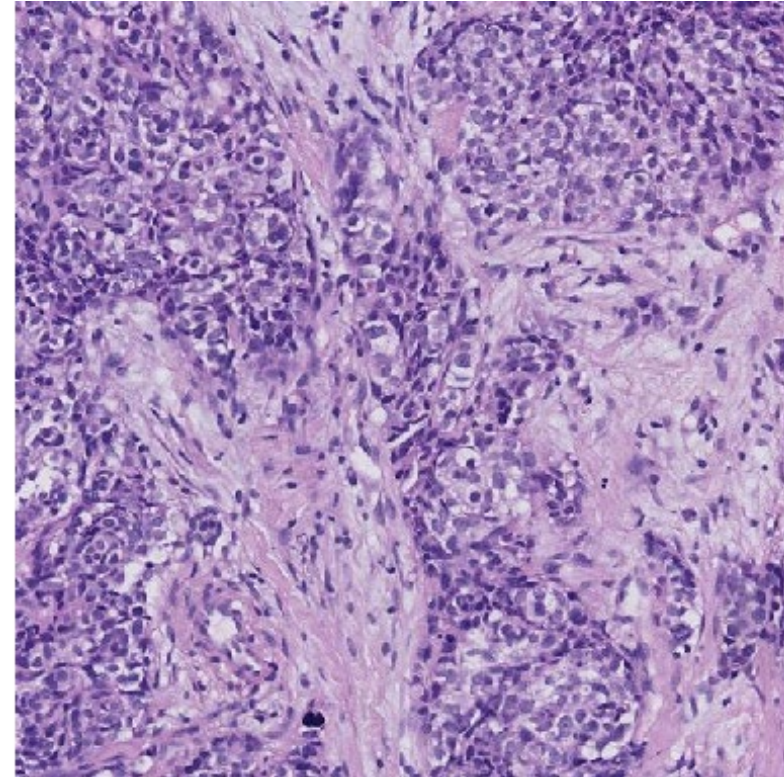
David L. Lawrence Convention Center  
The Westin Pittsburgh

**api**  
PATHOLOGY  
INFORMATICS

# Non-lymphoid cells may be confused for lymphocytes

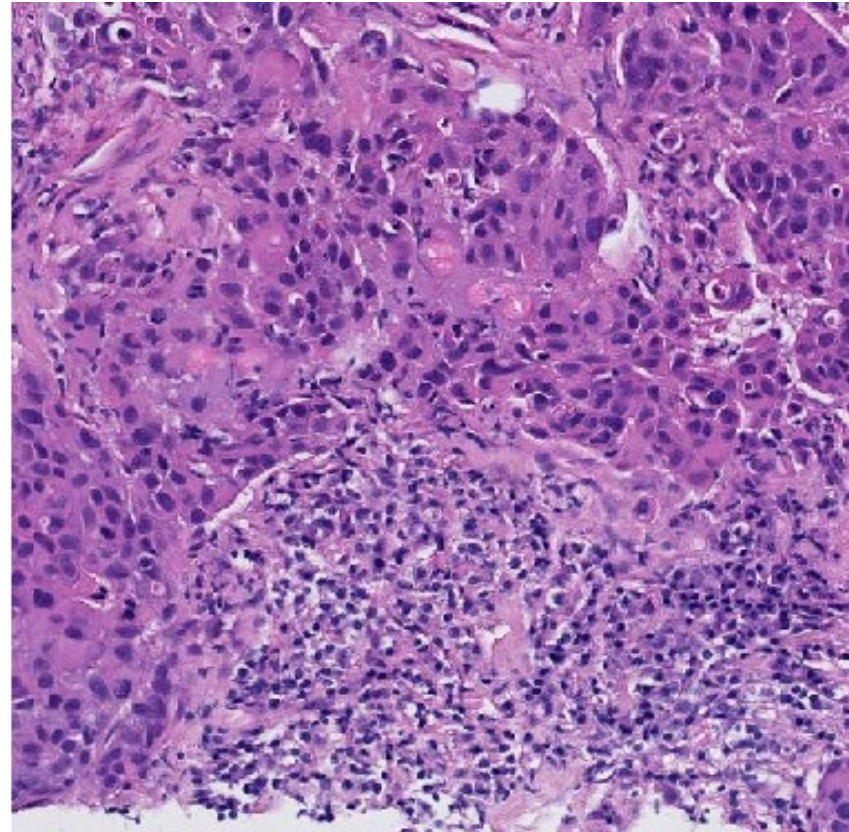


Fixation/preservation artifact



Cross-sectionally cut fibroblasts

Error in the percent tumor-associated stroma can affect the sTILs density



Sparingly distributed tumor cells may be more challenging to quantitate

