
Meeting PRIN2022
March 28th, 2025, 09.30-13.00
IOR, Via F. Chiesa 5, Bellinzona
Meeting Room 1 North West

Draft

PRIN2022: Targeting the intratumoral microbiota to reverse therapeutic resistance in lethal prostate cancers

Meeting objective: To provide an update on the current state of research and share the latest findings on the role of microbiota in cancer, with a particular focus on data from three research units.

11.30 – 12.15

Session 3: Intratumoral Microbiota and Pathology: The UNIVR Experience

Updates from Research Unit 3

Speaker: Matteo Brunelli

Topic: “RNAscope and ISH Technology and Intratumoral Microbiota”

Aim of session 3°

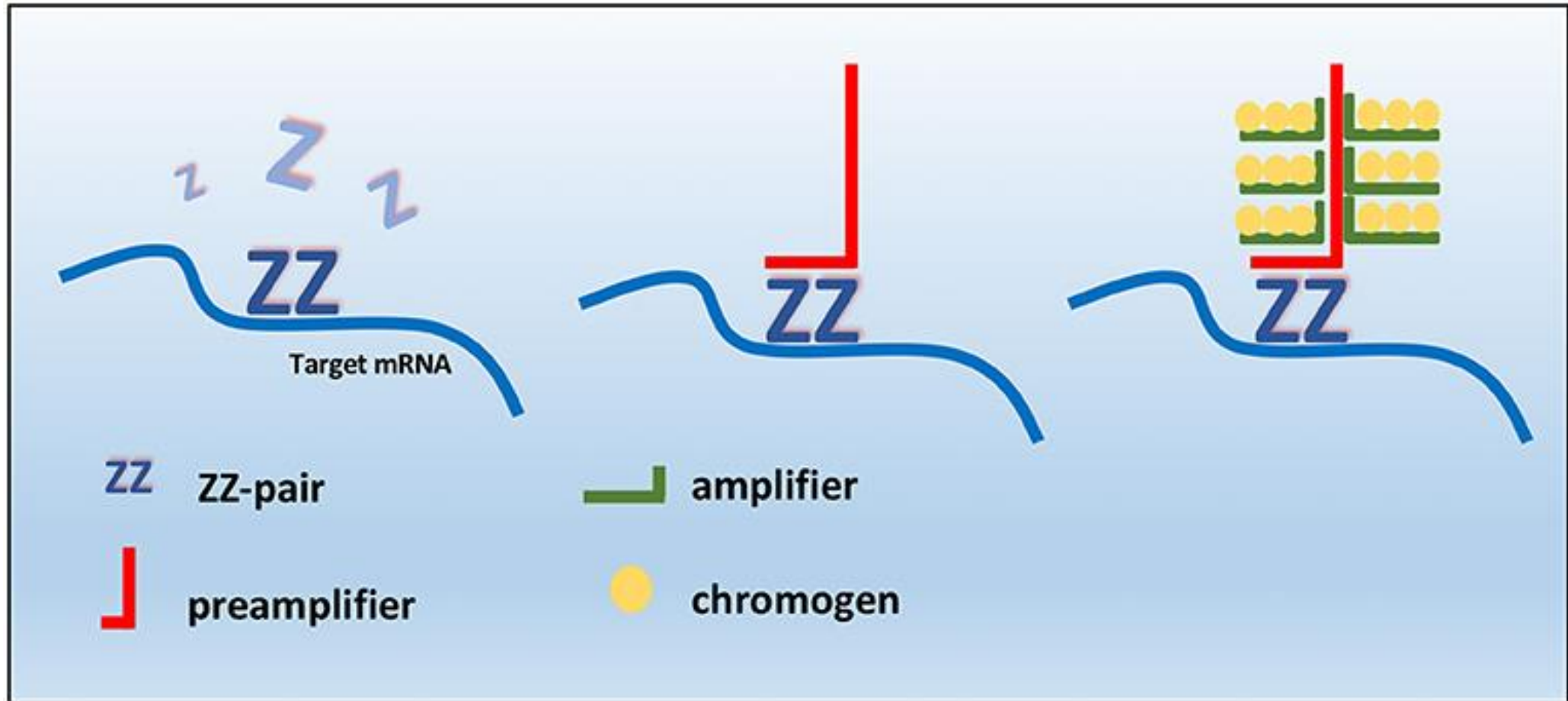
view of RNAscope technique
detection and visualization

cohort of studies – UNIVR experience
human prostate adenocarcinoma

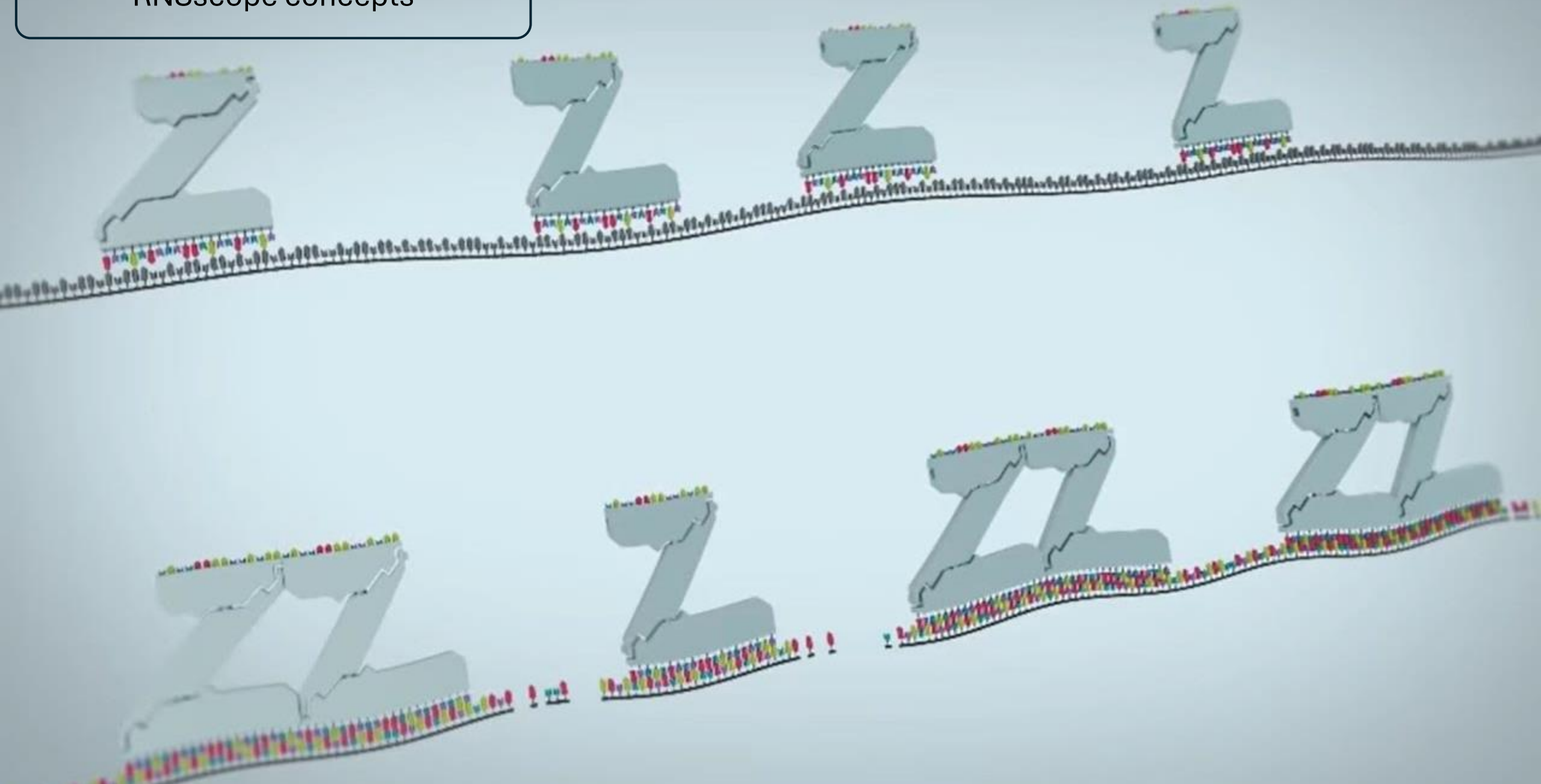
rRNA 16S detection in human adenocarcinoma and mouse models
results

criticisms and future directions

RNSscope concepts

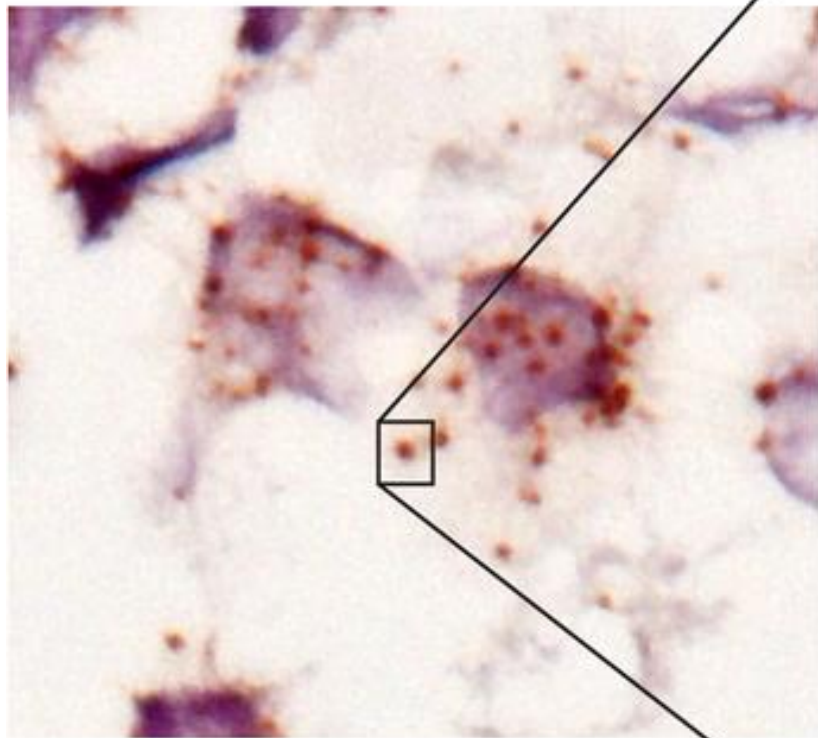


RNSscope concepts

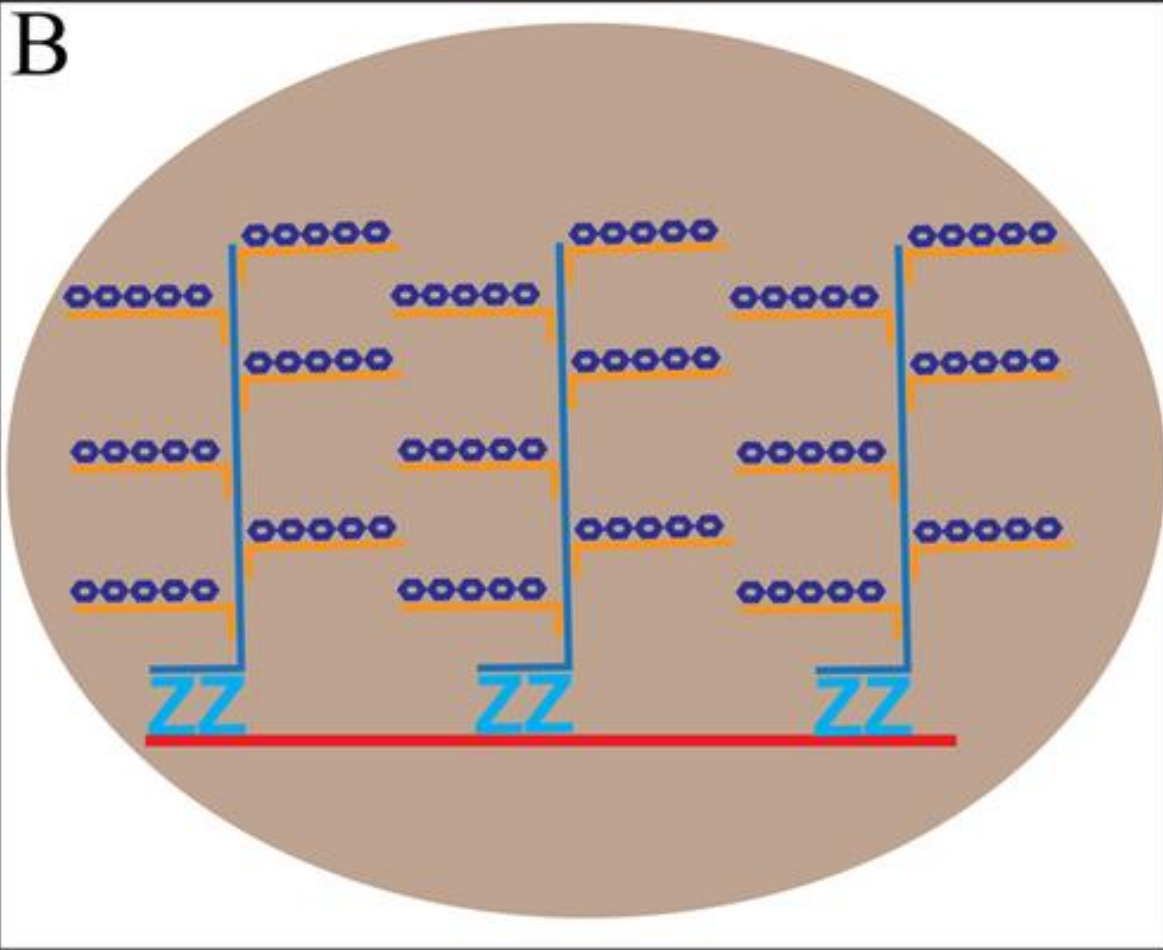


RNSscope concepts

A



B



— Target Transcript

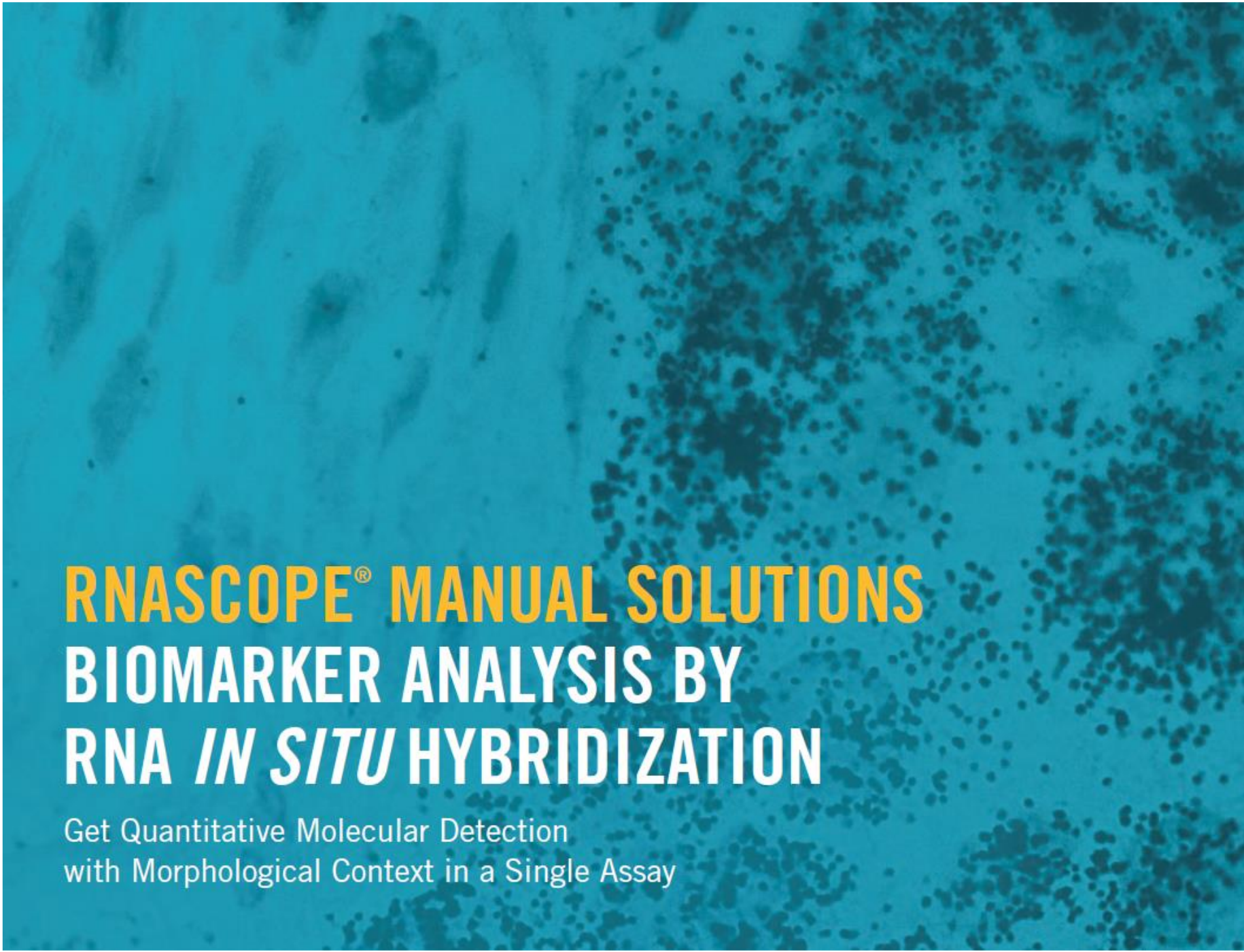
ZZ Target Probe

Pre-amplifier

— Amplifier

⬡ Detection Probe (+HRP)

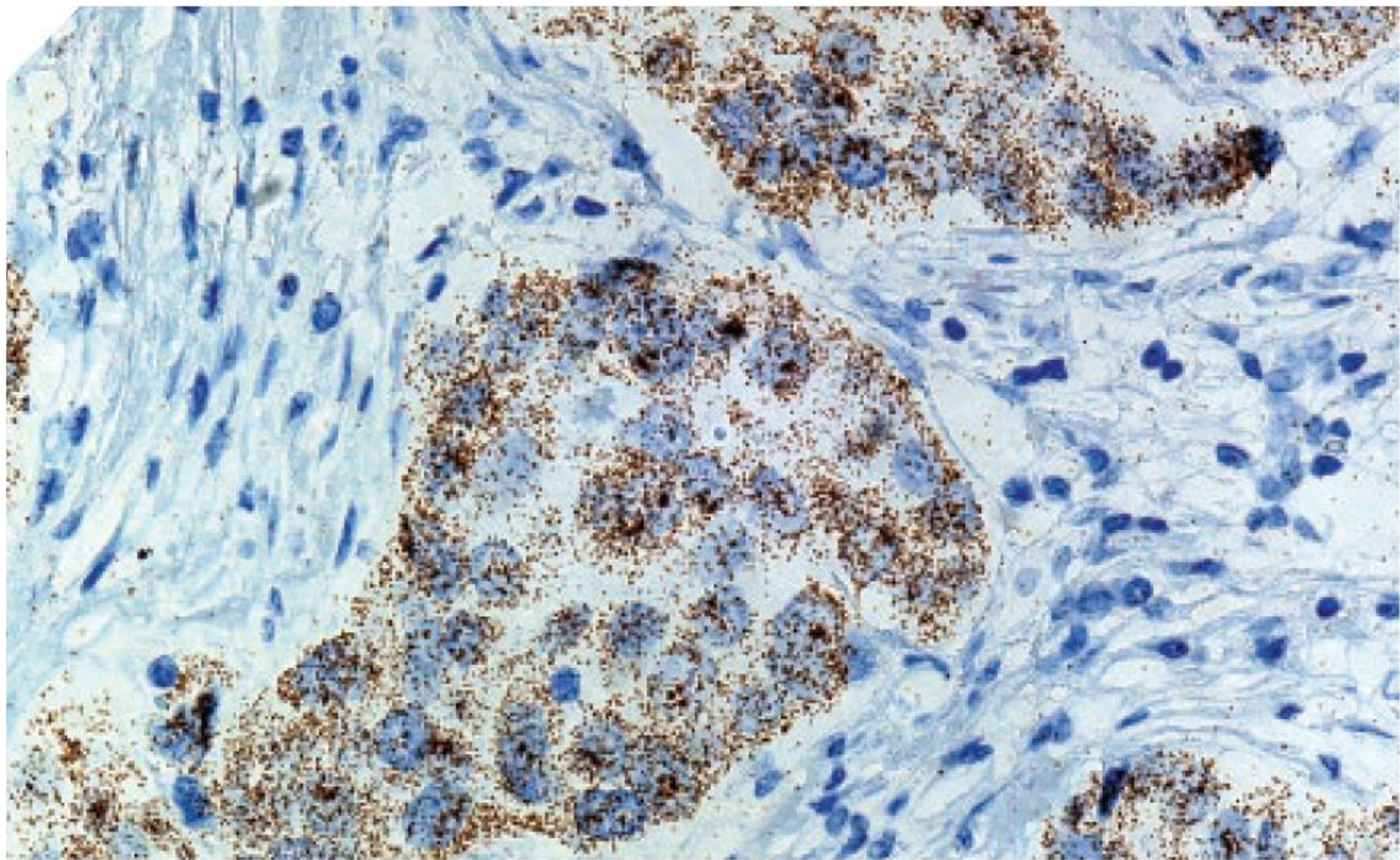
● DAB Puncta

The background of the slide is a microscopic image showing a dense population of cells. The cells are stained with a blue dye, likely DAPI, which highlights the nuclei. Overlaid on this are numerous small, dark, punctate signals, which represent the RNA in situ hybridization results. These signals are distributed throughout the field of view, with some areas showing higher density than others.

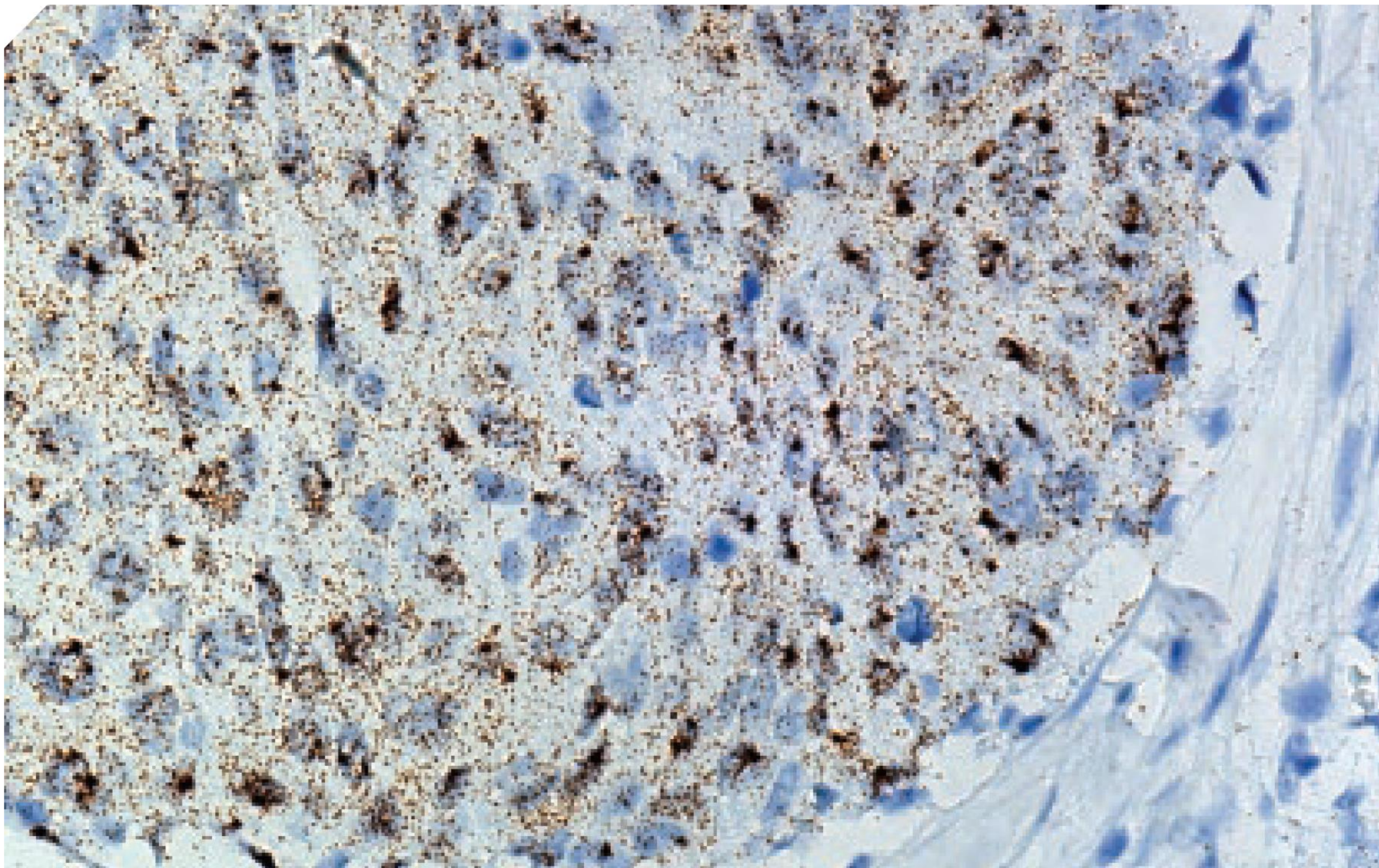
RNASCOPE[®] MANUAL SOLUTIONS **BIOMARKER ANALYSIS BY** **RNA *IN SITU* HYBRIDIZATION**

Get Quantitative Molecular Detection
with Morphological Context in a Single Assay

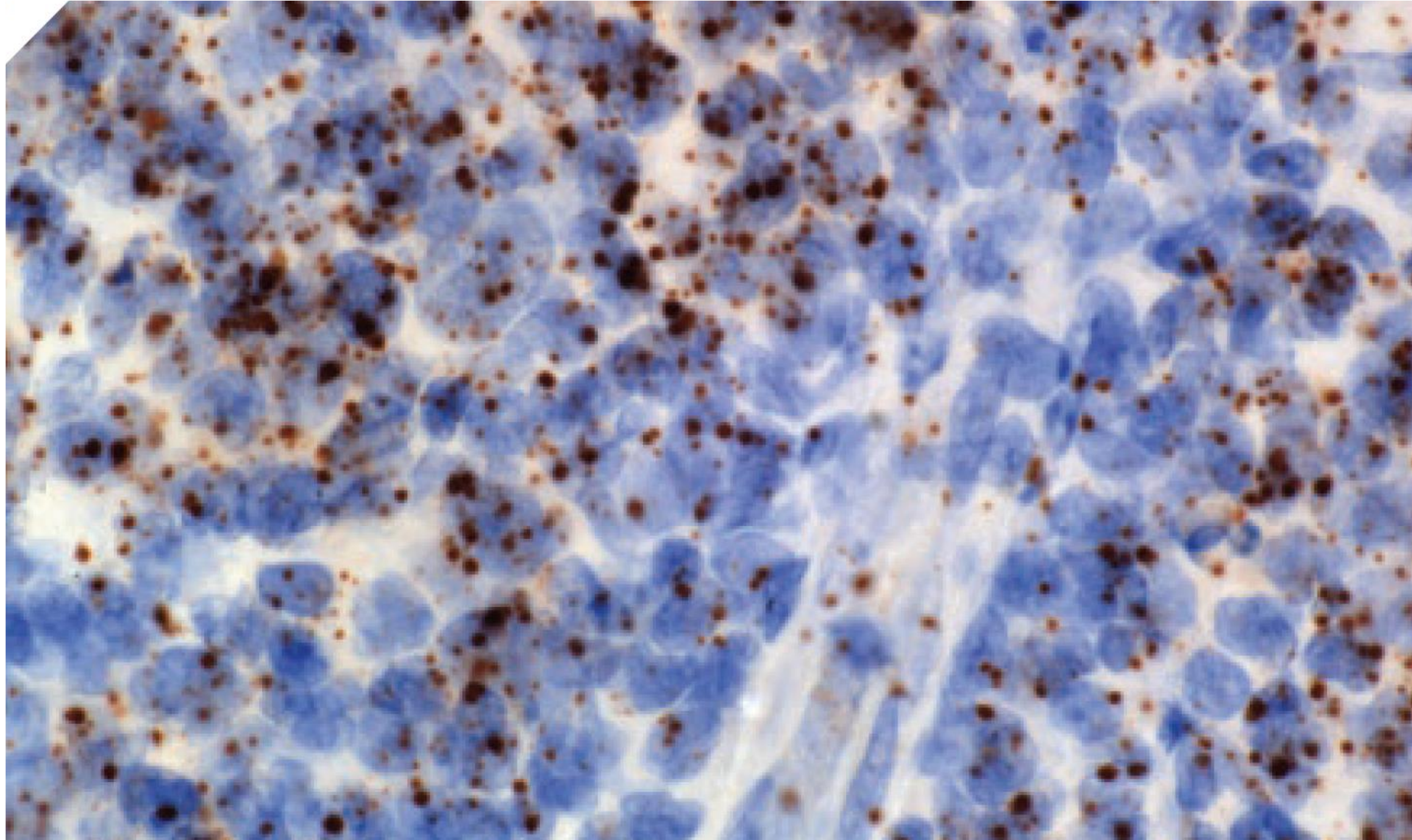
HER-2



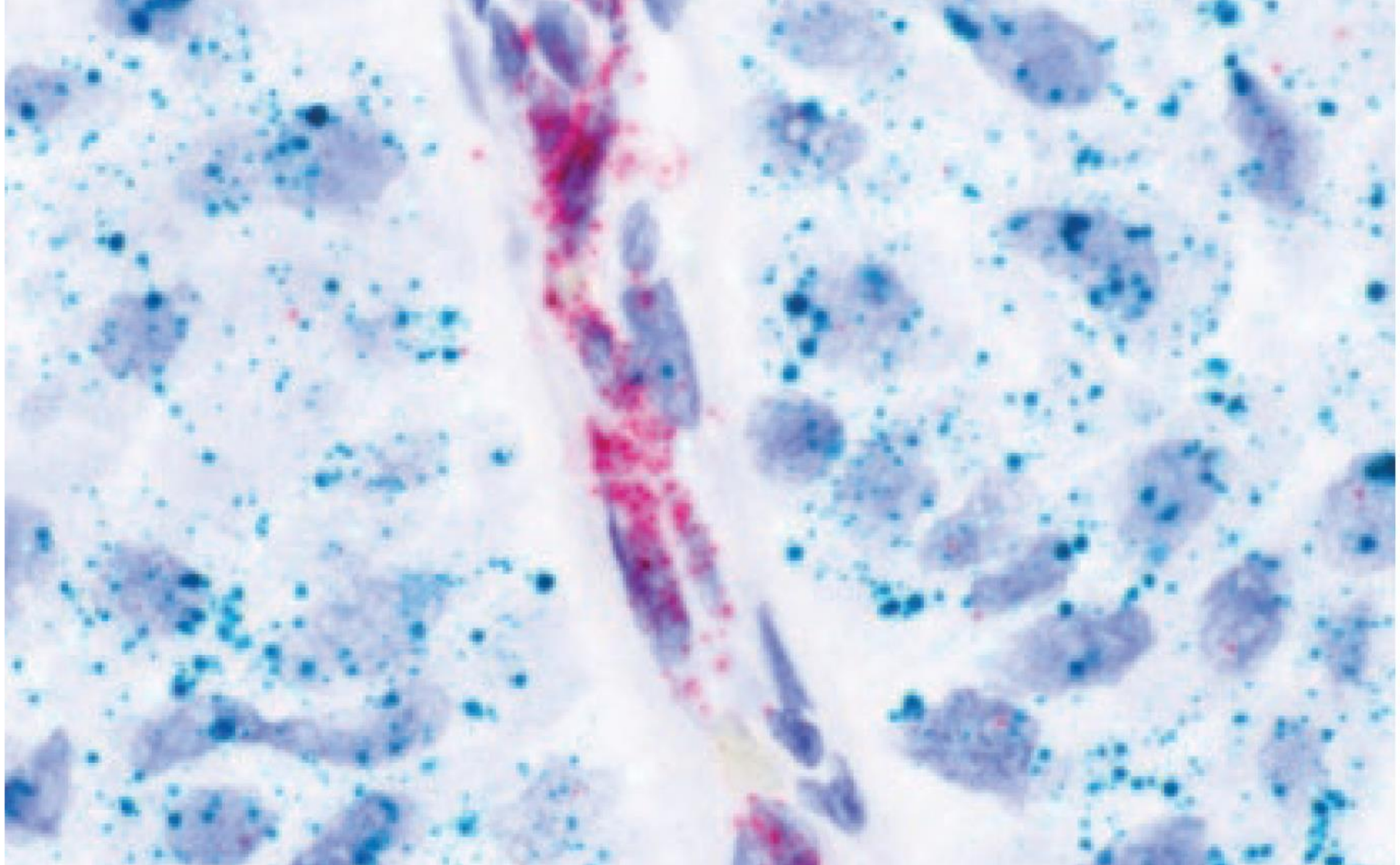
HPV
E6/E7



PTEN



EPCAM/EGFR



VISUALIZE

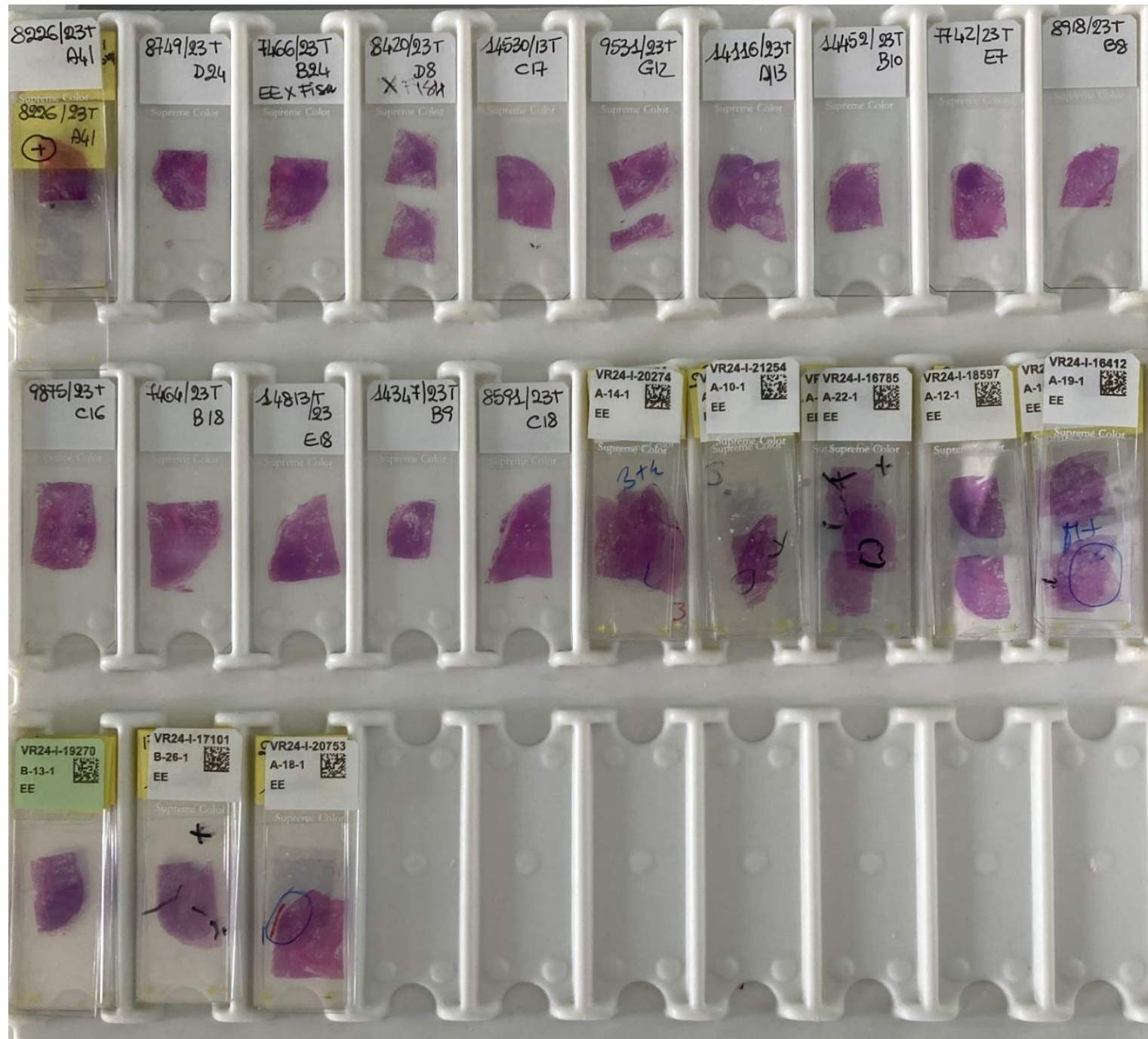
**EACH PUNCTATE DOT SIGNAL
REPRESENTS A SINGLE
TARGET RNA MOLECULE AND
CAN BE VISUALIZED WITH
A MICROSCOPE**

UNIVR cohort – human prostate adenocarcinoma

castration resistant prostate adenocarcinoma

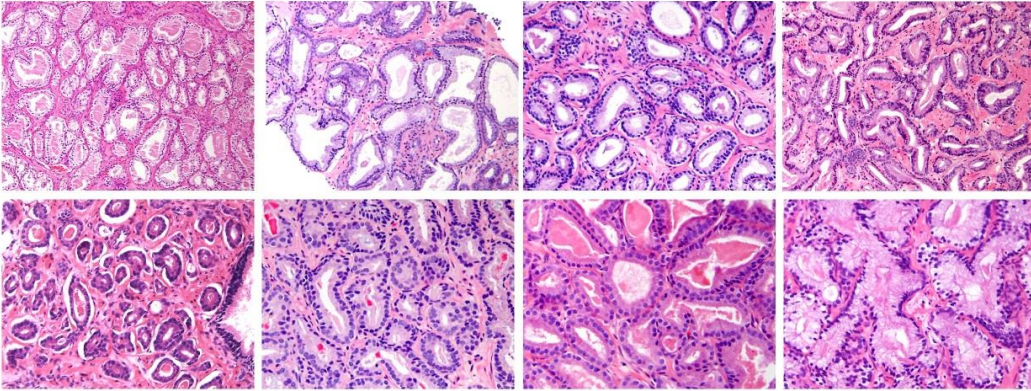


around 50 haematoxylin and eosin glass slides per
single case

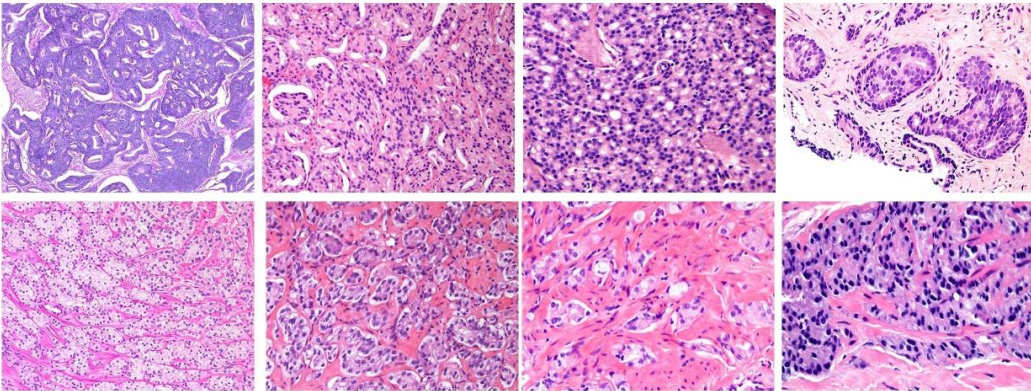


human prostate adenocarcinoma
University of Verona

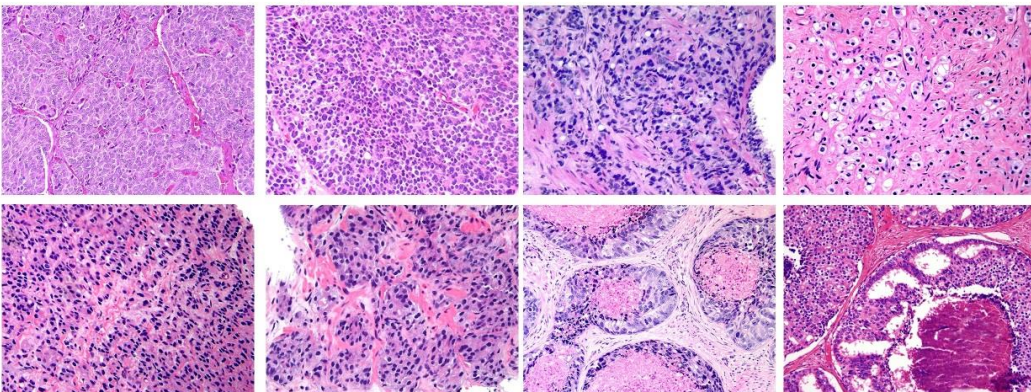
Discrete Well-formed Glands (Gleason Patterns 1-3)



Cribriform/Poorly-formed/Fused Glands (Gleason Pattern 4)



Sheets/Cords/Single Cells/Solid Nests/Necrosis (Gleason Pattern 5)



WHO 2022

3+3 = GRADE GROUP 1

3+4 = GRADE GROUP 2

4+3 = GRADE GROUP 3

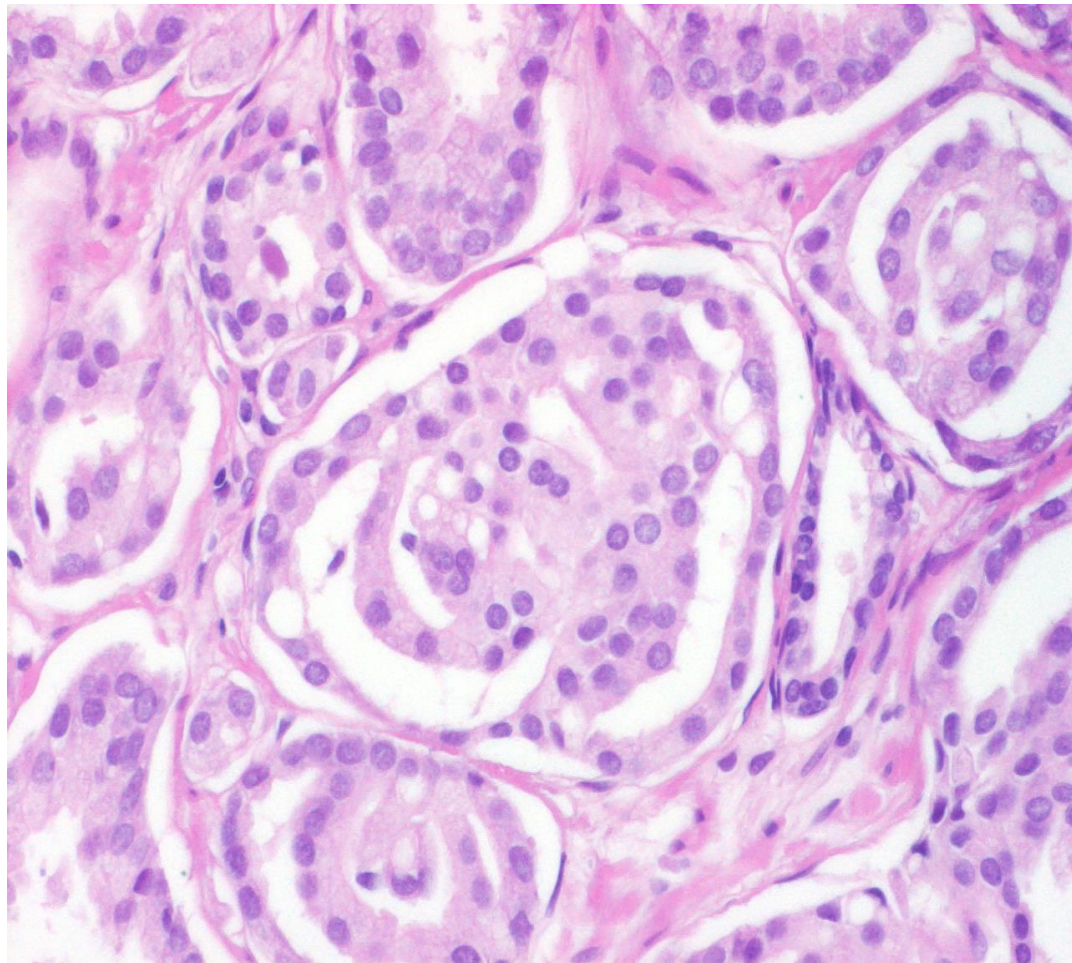
4+4 = GRADE GROUP 4

4+5; 5+4; 5+5 = GRADE GROUP 5

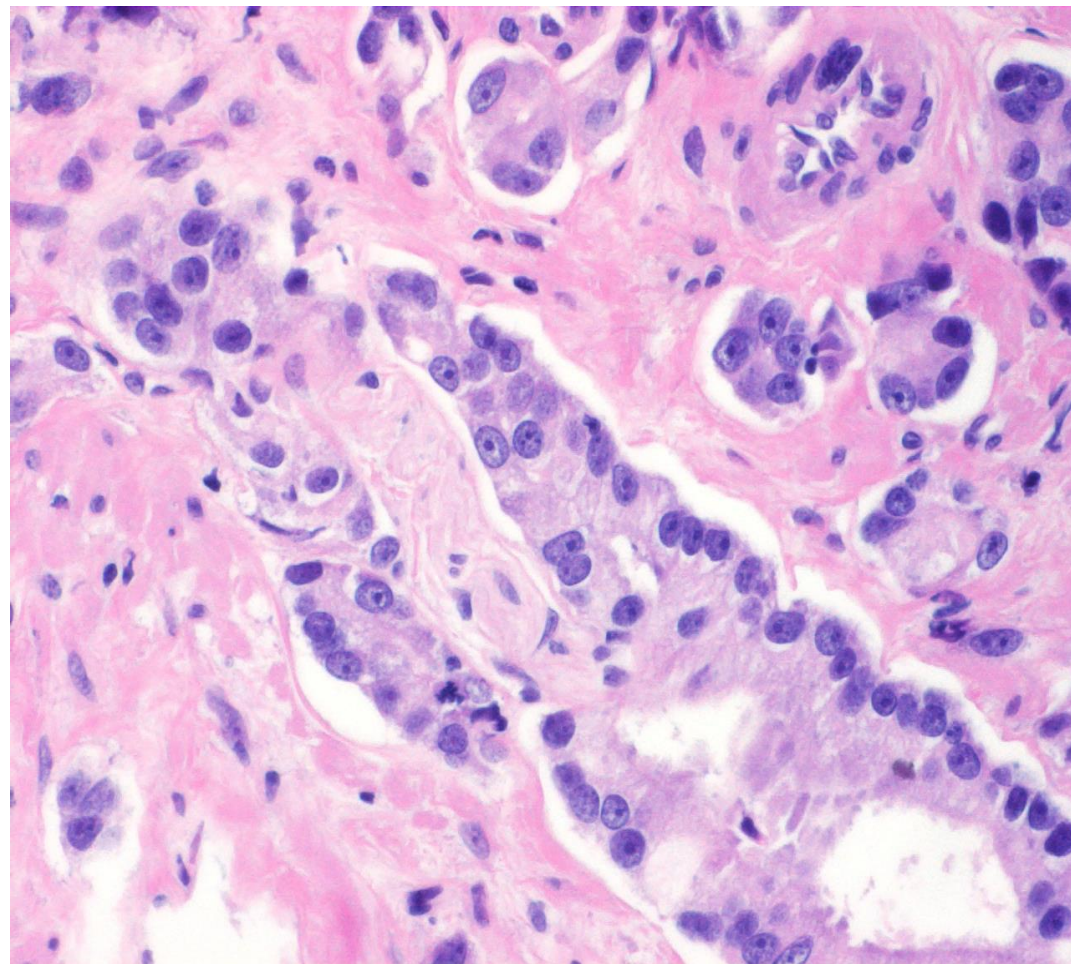


human prostate adenocarcinoma

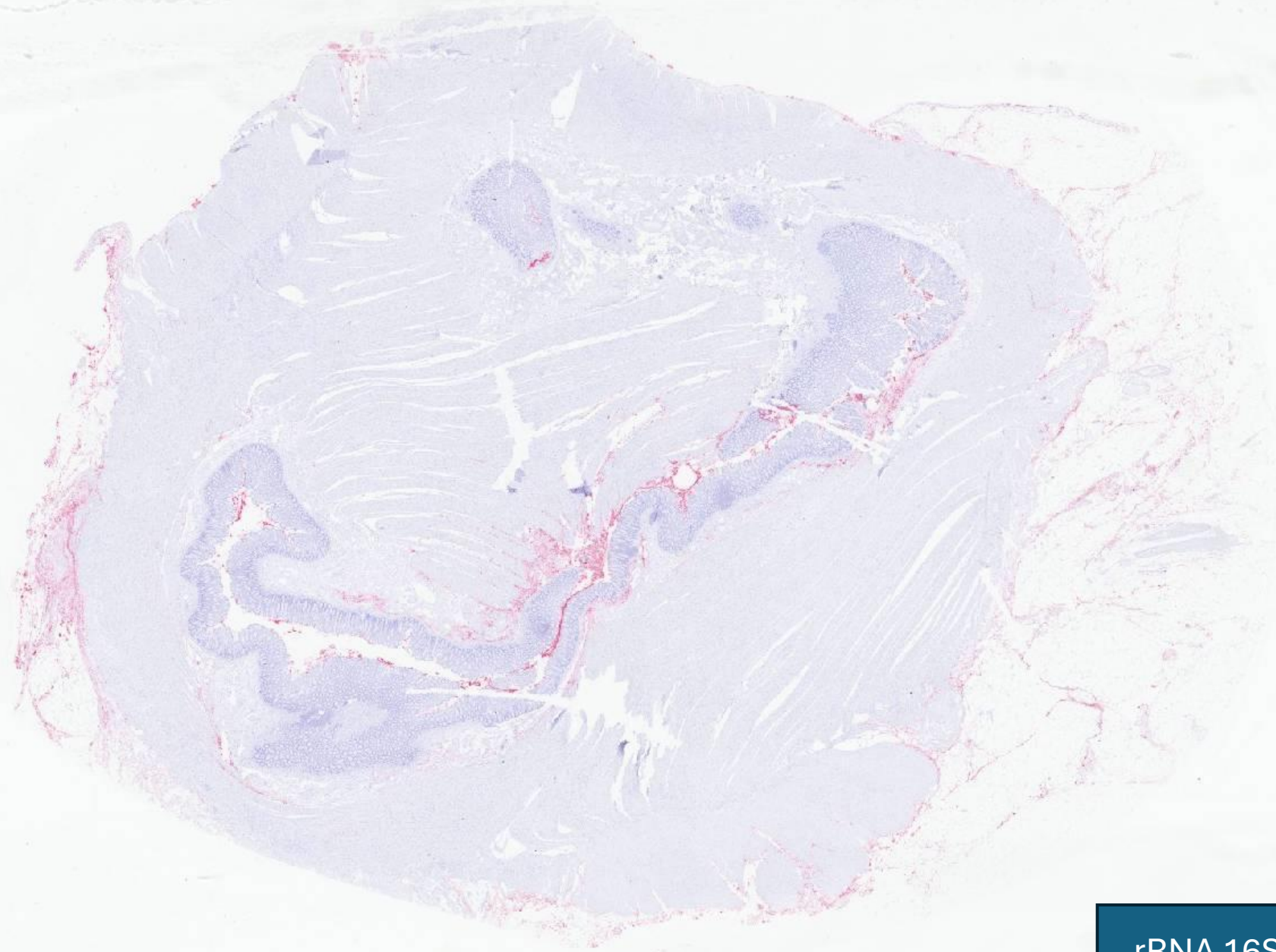
selection of paraffin blocks



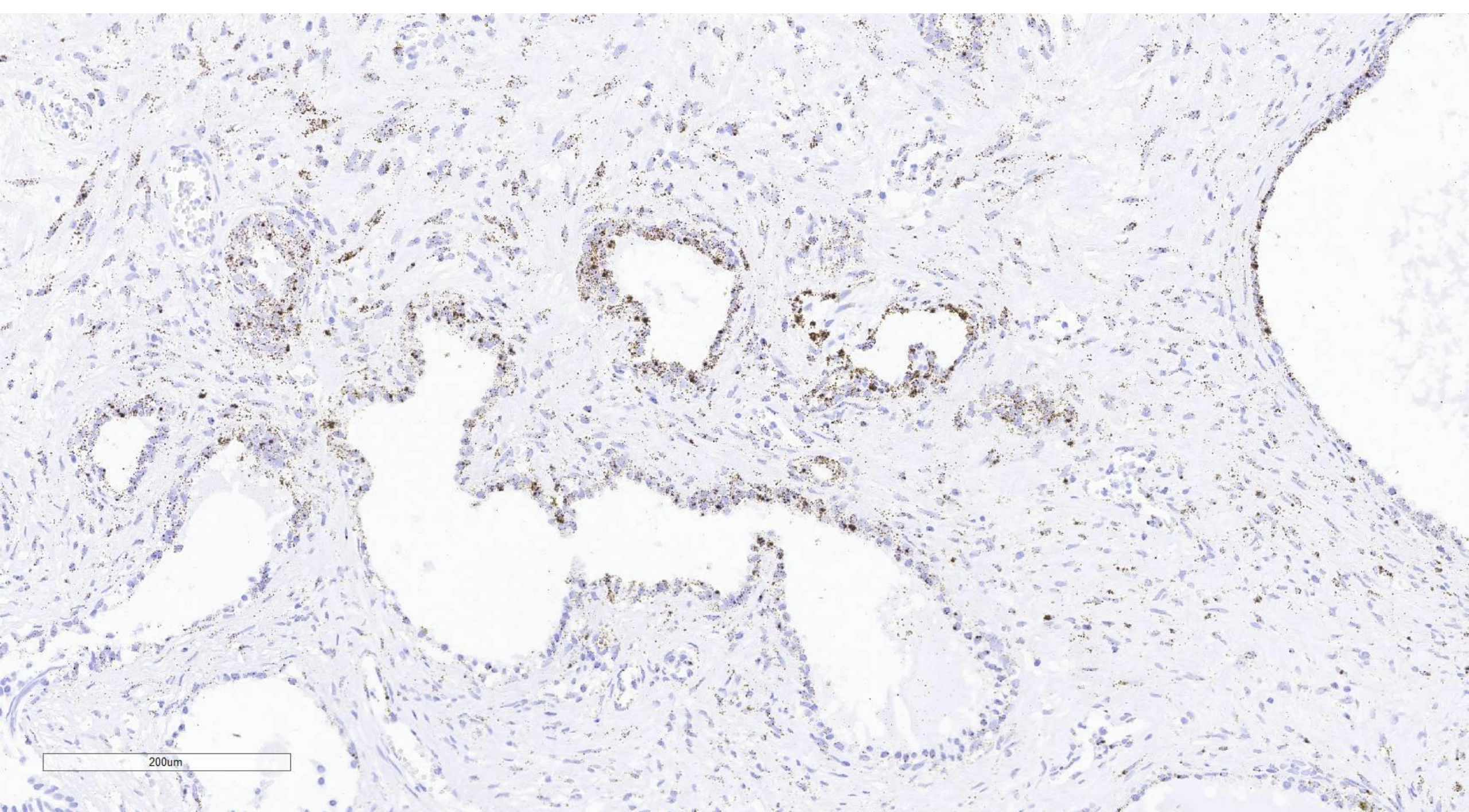
cribriform 4+4 Gleason



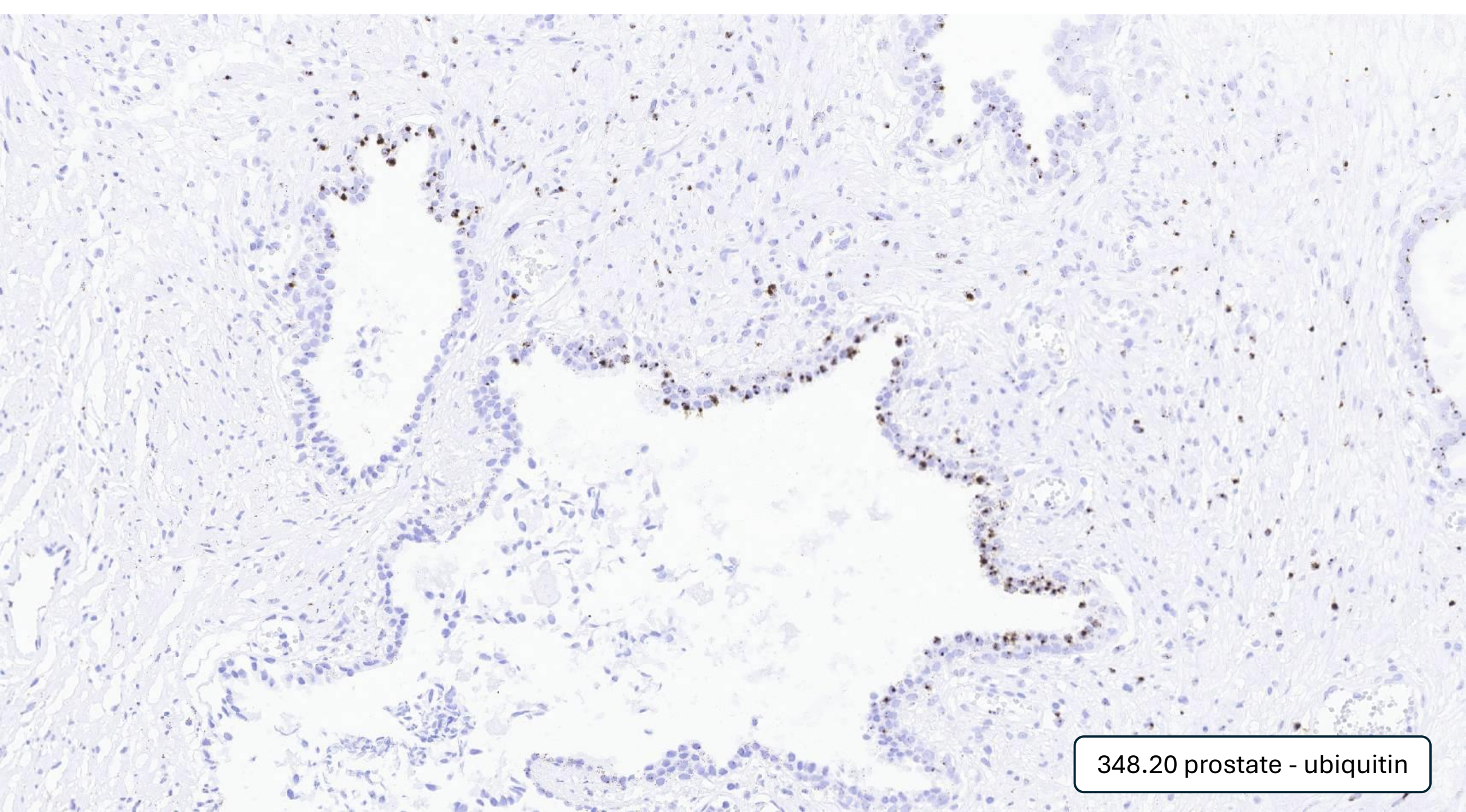
nested and single cells 4+5
Gleason



rRNA 16S - bowel as control



200um

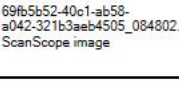


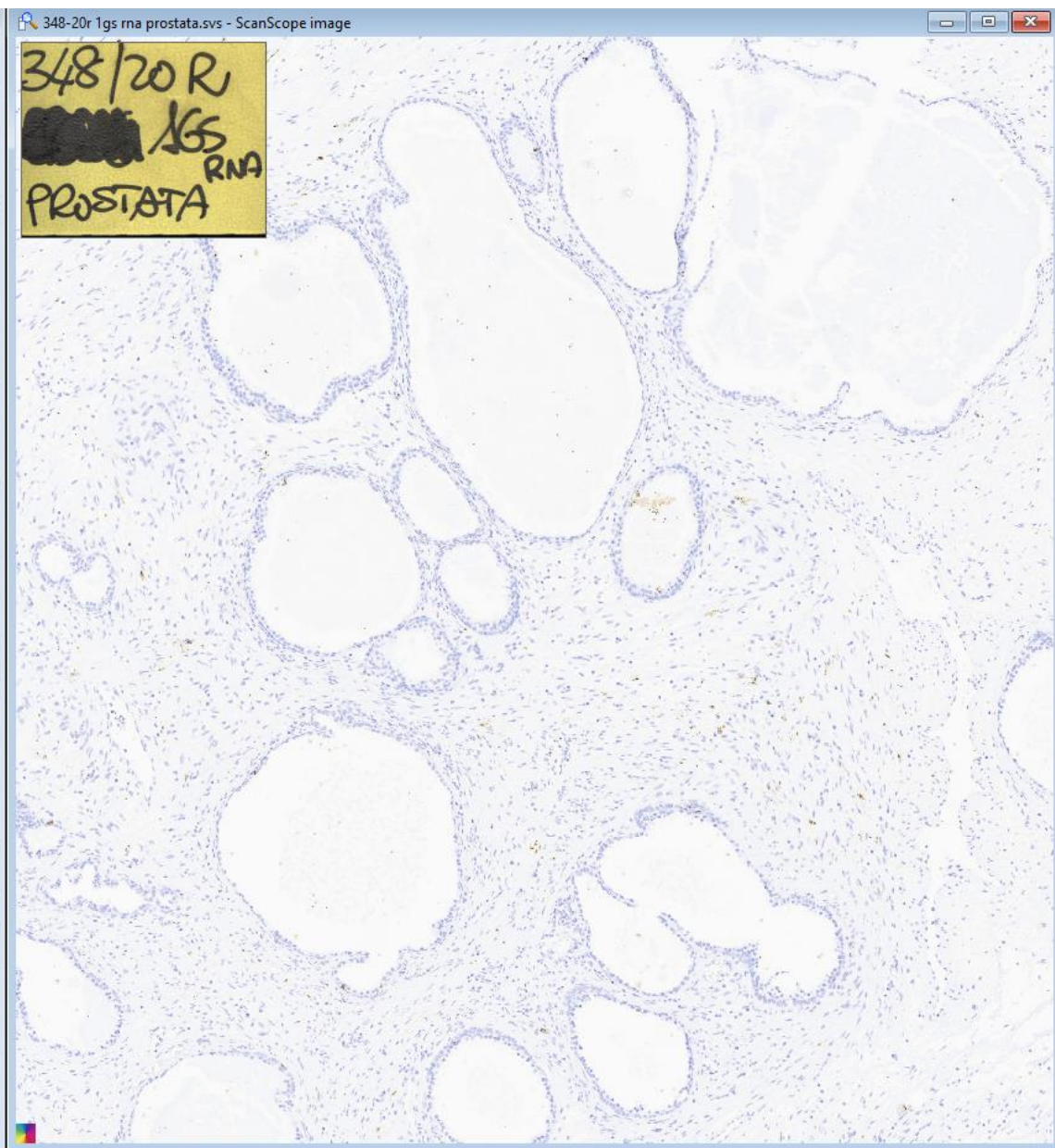
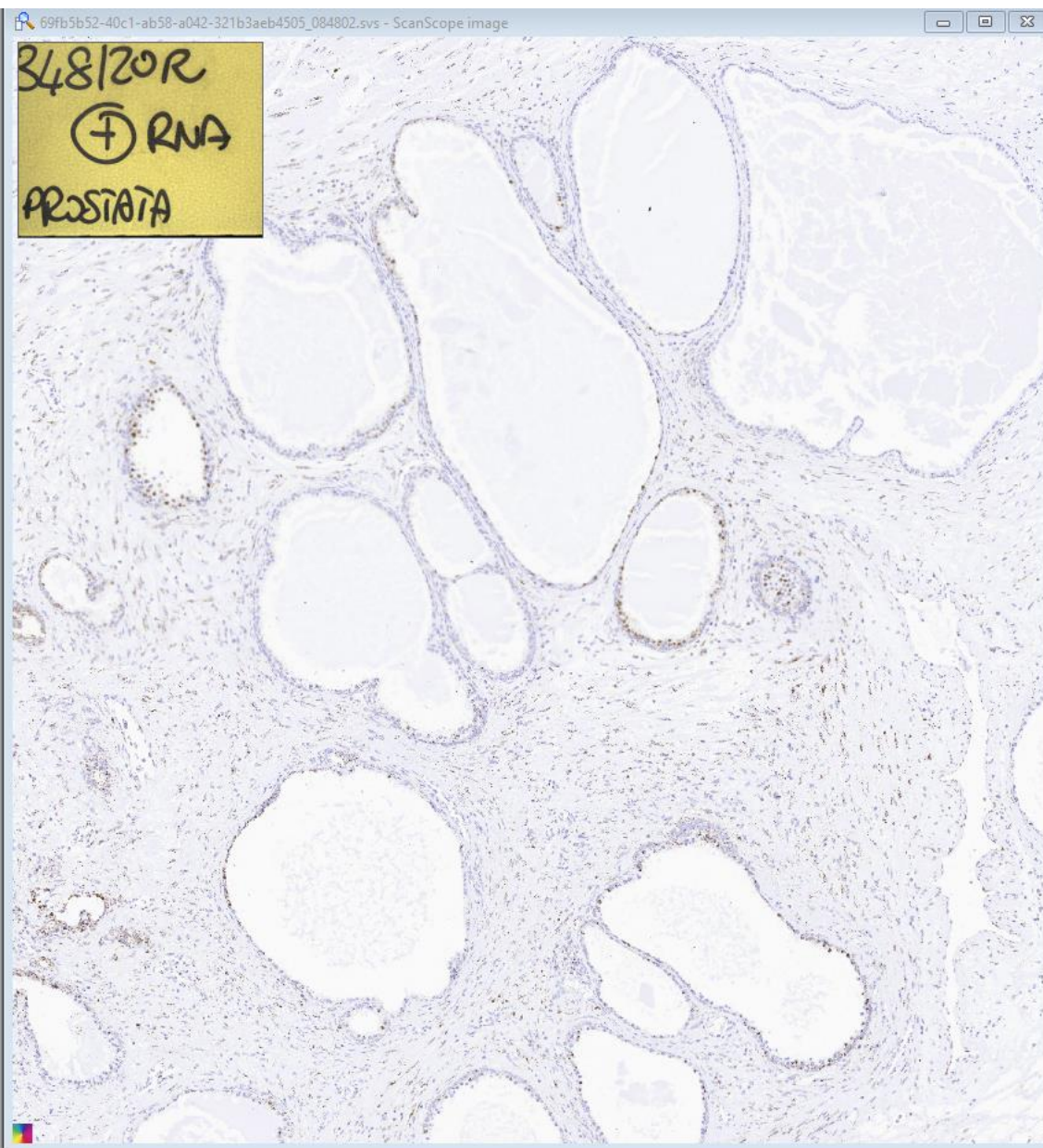
348.20 prostate - ubiquitin

348-20r 1gs rna prostata.svs - ScanScope image

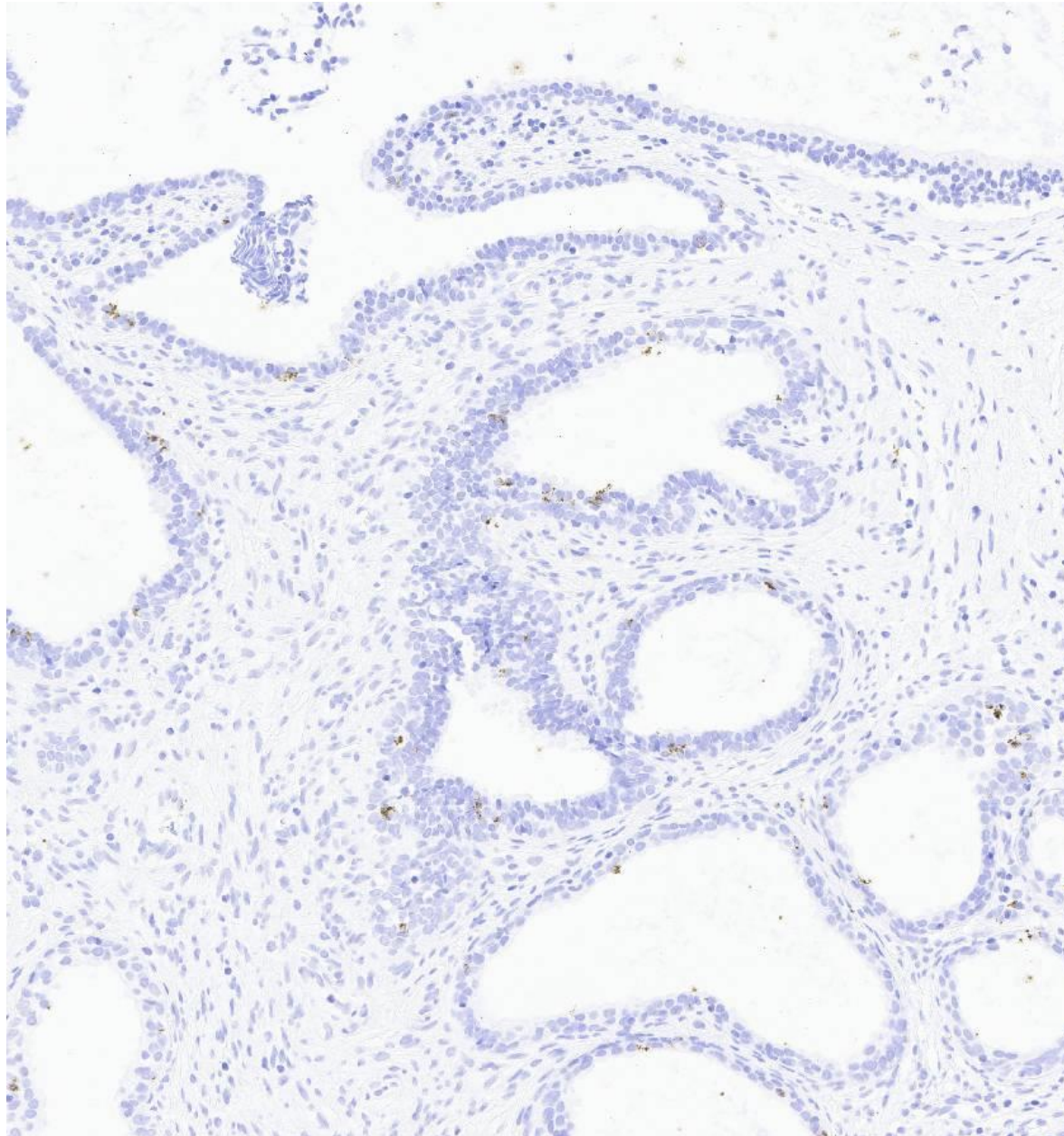


69fb5b52-40c1-ab58-a042-321b3aeb4505_084802.svs - ScanScope image

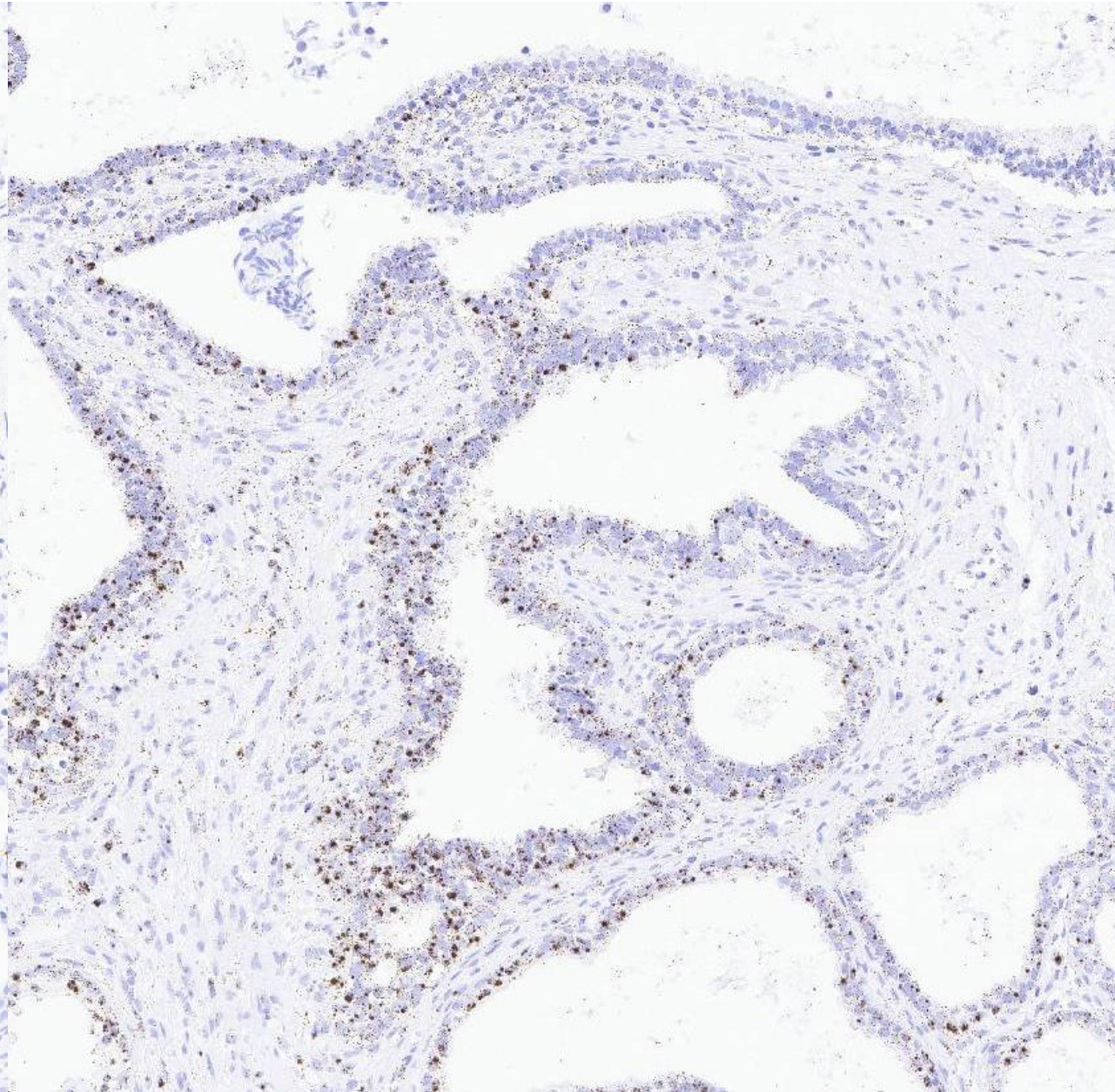




rRNA 16S prostate

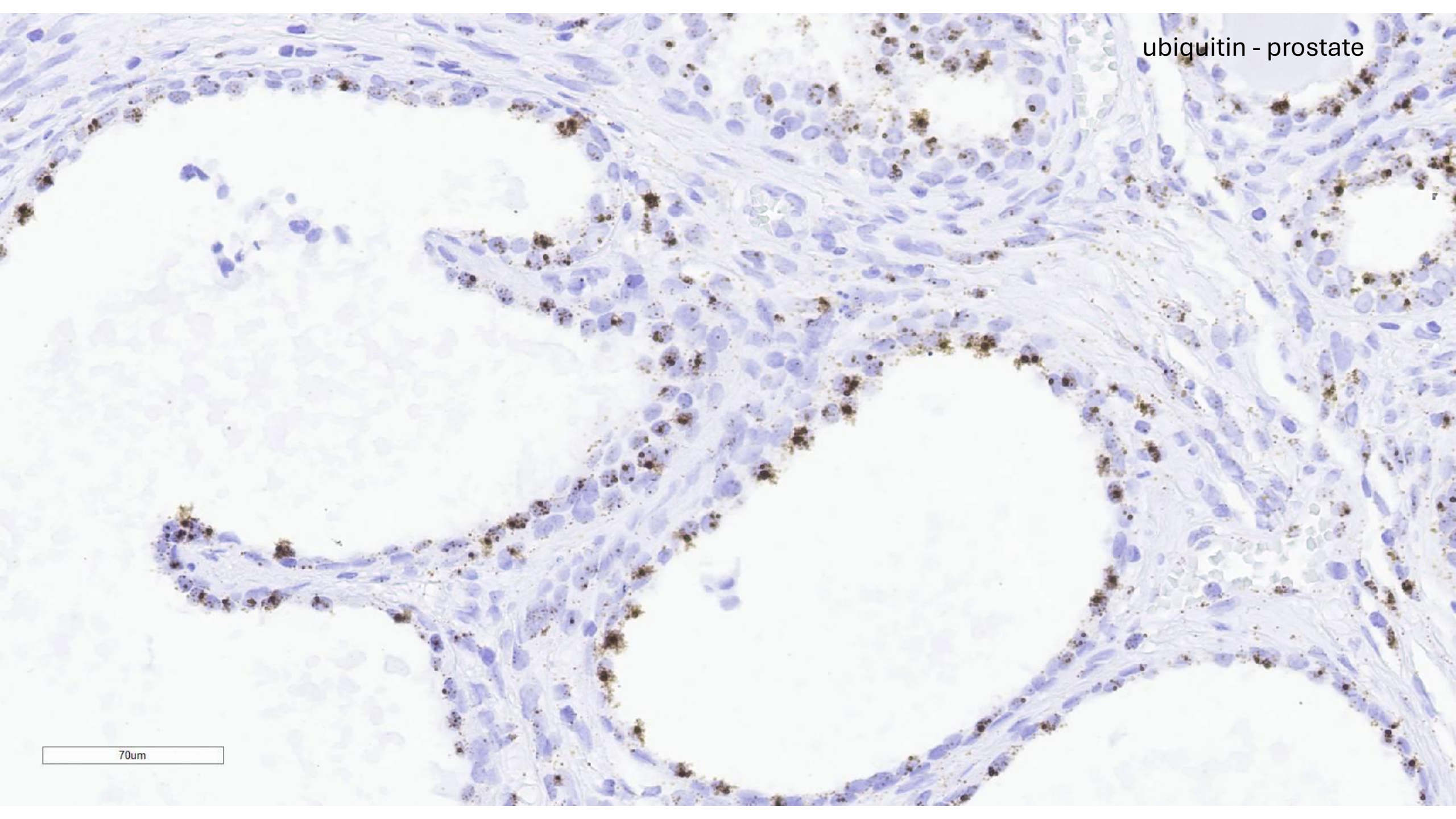


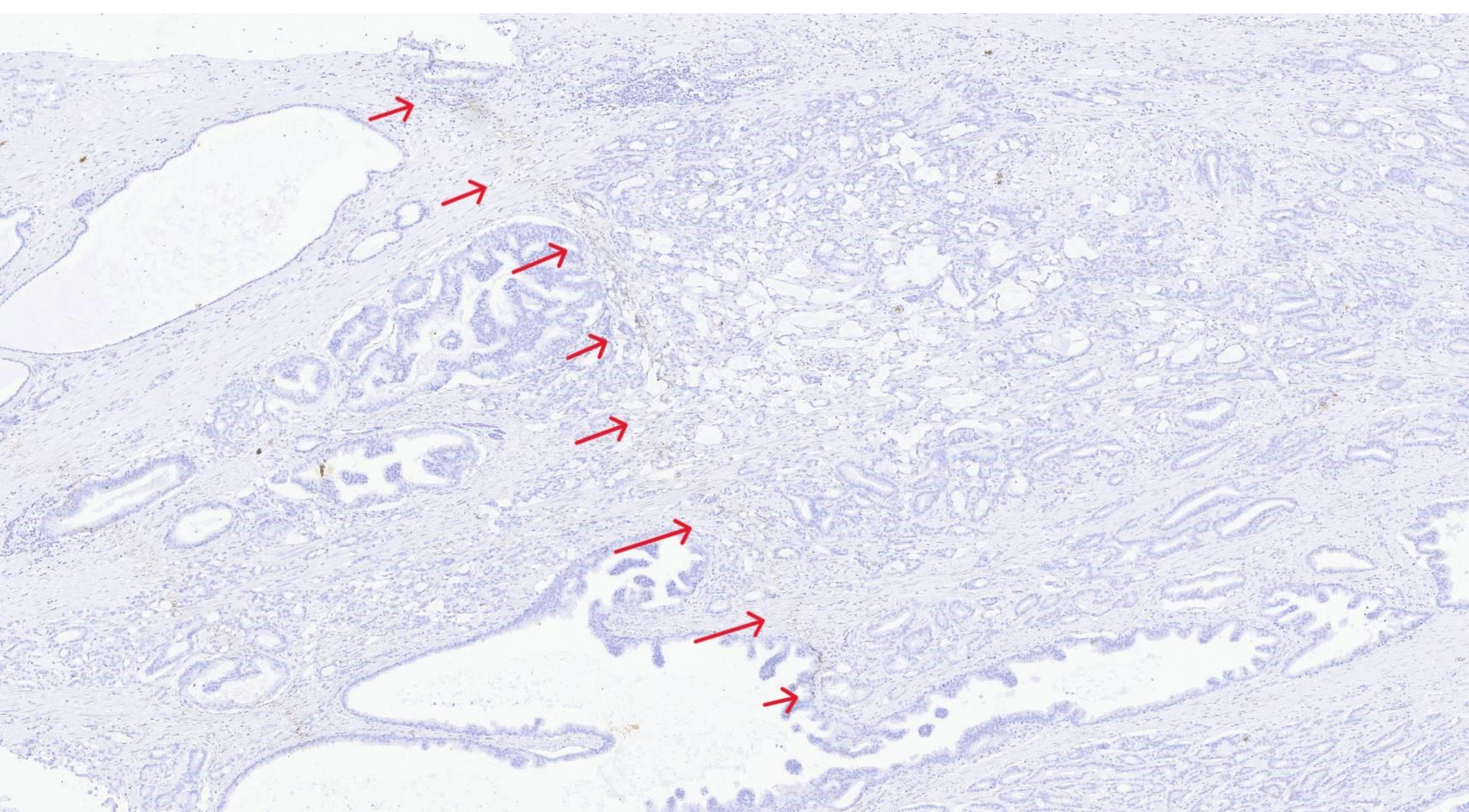
Ubiquitin - prostate



ubiquitin - prostate

70um





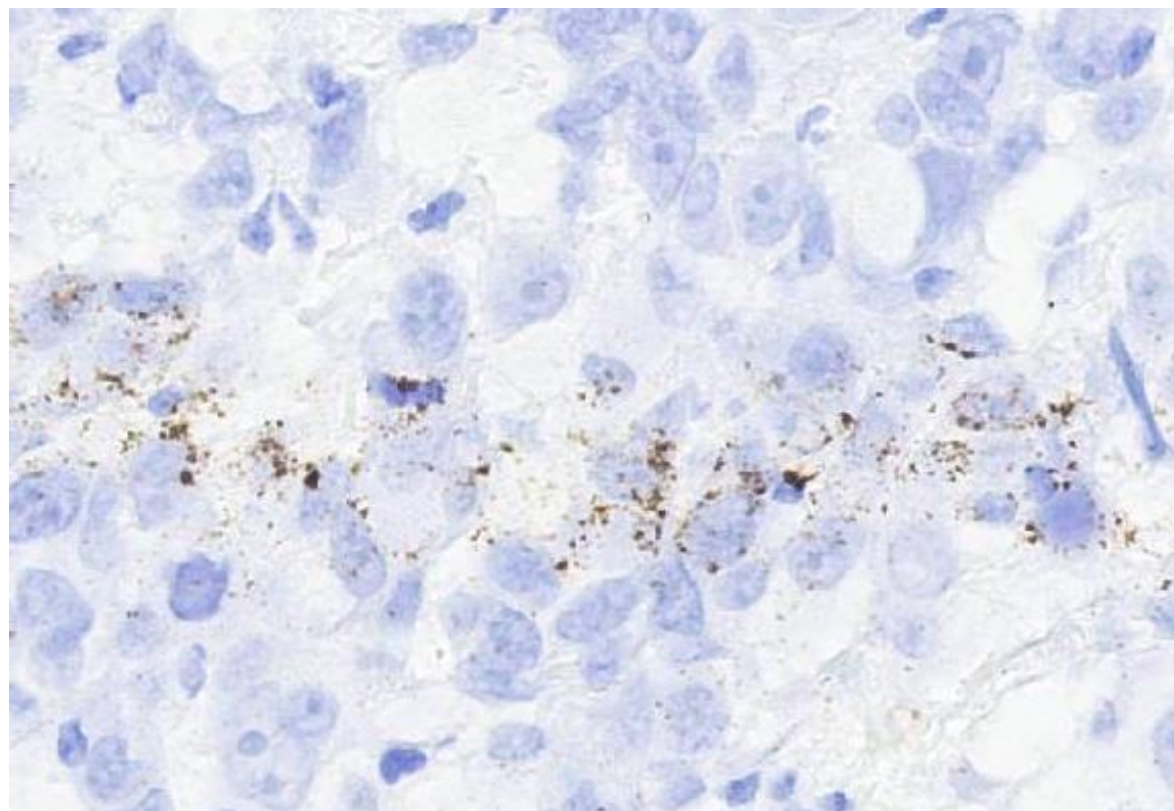
8749/23 +
D24

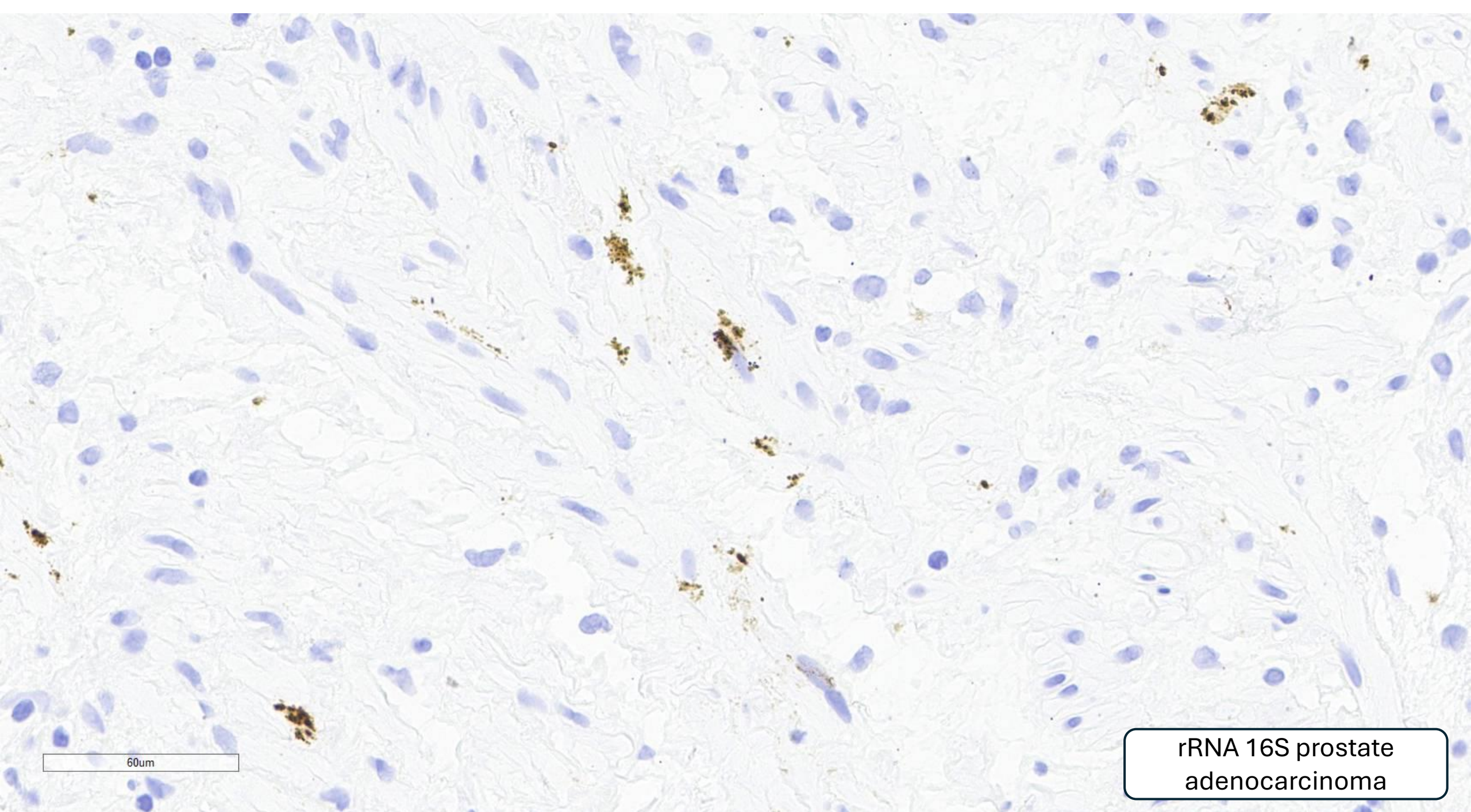
Supreme Color



26221

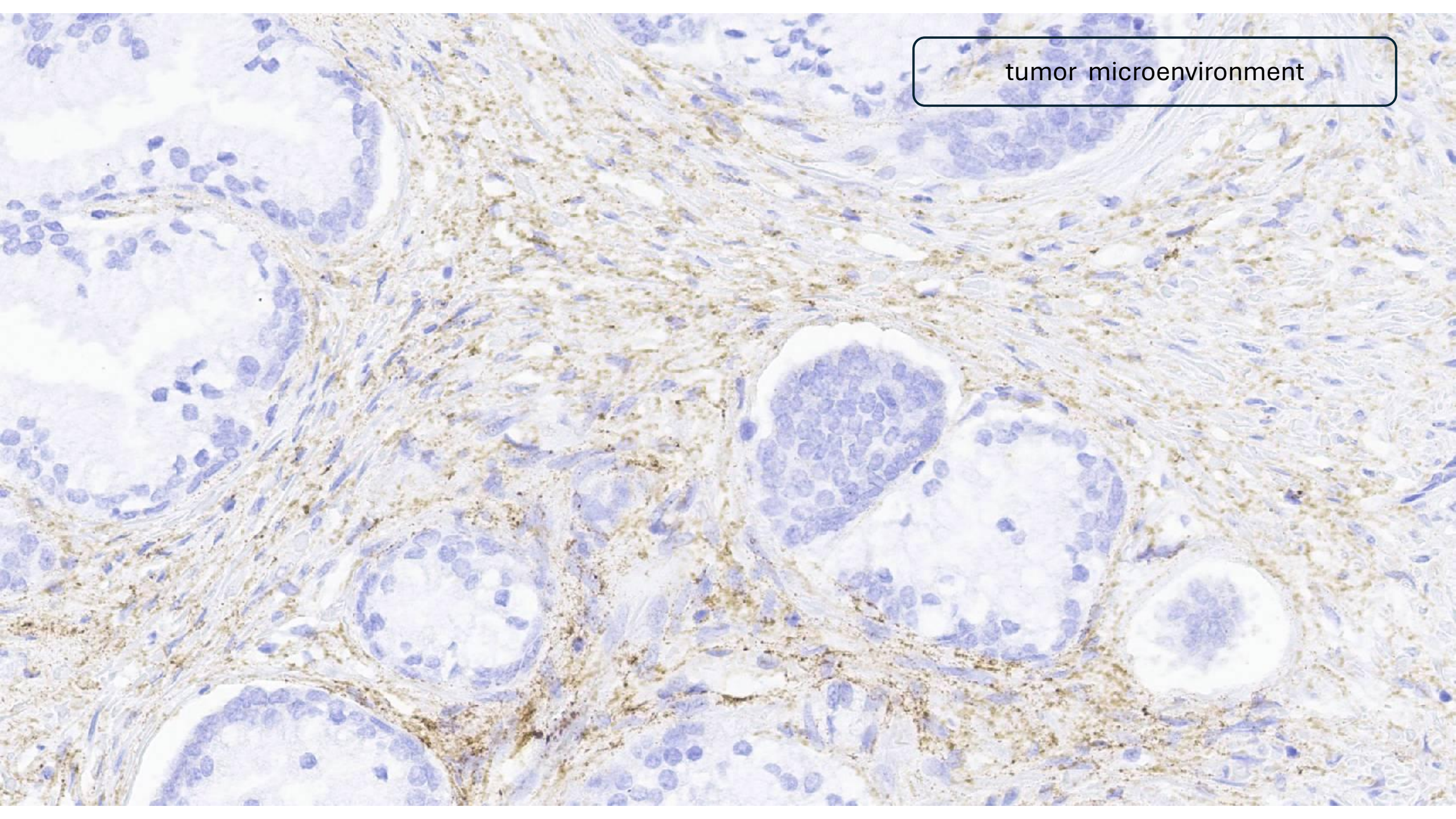
rRNA 16S in human prostate
adenocarcinoma





60um

rRNA 16S prostate
adenocarcinoma

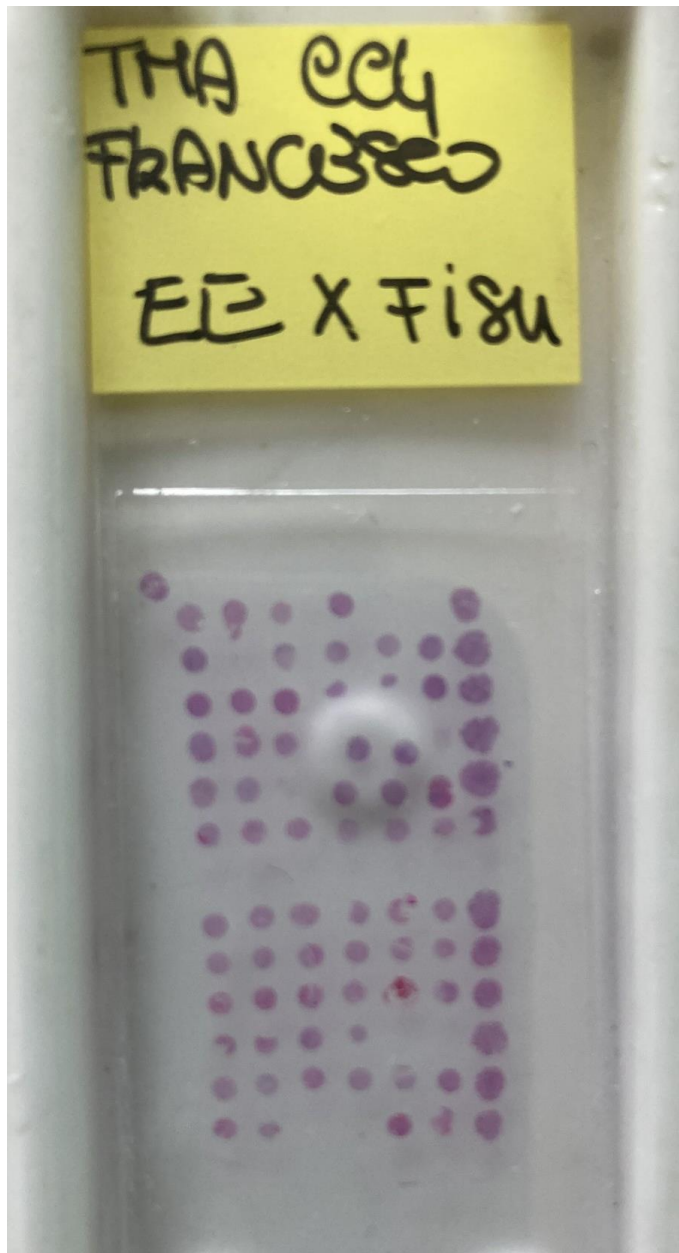


tumor microenvironment

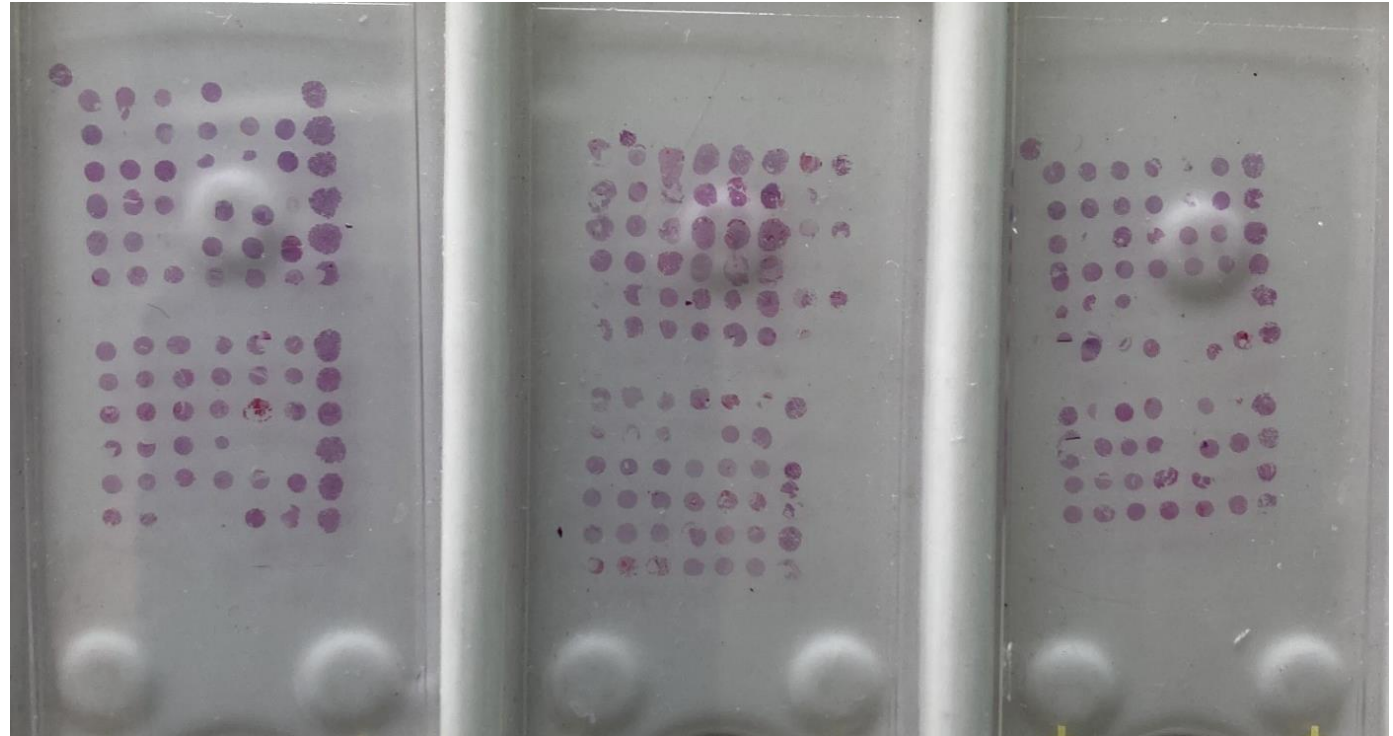
This histological image shows a section of tissue with several glandular structures. The glands are lined by a layer of cells with dark blue nuclei. The surrounding stroma is filled with a dense network of brown-stained fibers, likely representing the extracellular matrix or a specific marker in the tumor microenvironment. The overall architecture suggests a complex interaction between the tumor cells and their surrounding environment.

tumor microenvironment

80um

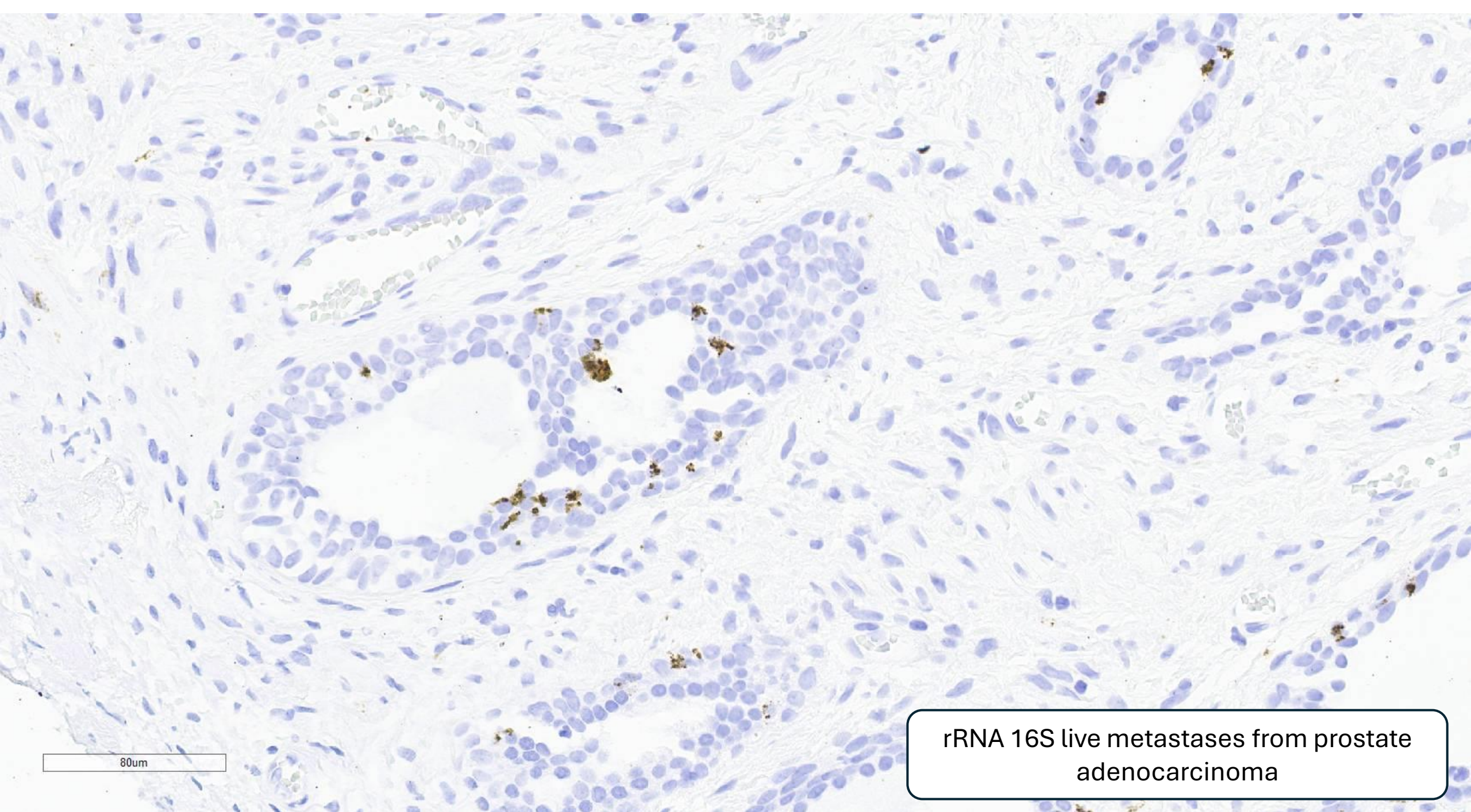


n. 3 tissue microarrays (TMAs)



1.6 mm per core

252 cores from human prostate adenocarcinoma



80um

rRNA 16S live metastases from prostate
adenocarcinoma

rRNA 16S has been observed in:

- 1) 23% (16/72) castration resistant human prostate adenocarcinoma
- 2) 11% (2/22) of lymph-nodal metastases
- 3) 14% (3/21) visceral metastases

Bellinzona – mouse models

2

ORAL BACTERIA

NP15 ABX

NP15 ABX 32349 0 SHIG
NP15 ABX 32349 1 SHIG
NP15 ABX 32349 2 SHIG
NP15 ABX 32349 5 SHIG
NP15 ABX 32352 1 HF
NP15 ABX 32352 4 HF
NP15 ABX 32352 5 HF
NP15 ABX 28279 1 PBS
NP15 ABX 28279 2 PBS
NP15 ABX 28279 3 PBS
NP15 ABX 32353 0 SHIG
NP15 ABX 32353 2 SHIG
NP15 ABX 32353 3 SHIG
NP15 ABX 32351 0 HF
NP15 ABX 32351 1 HF
NP15 ABX 32351 2 HF
NP15 ABX 32351 3 HF
NP15 ABX 28007 1 PBS
NP15 ABX 28007 3 PBS
NP15 ABX 28007 4 PBS
NP15 ABX 28007 5 PBS

2 RNA scope

INTRATUMORAL BACTERIA

NP14

NP14 26482 0 HF
NP14 25253 1 HF
NP14 26482 2 HF
NP14 26485 3 HF
NP14 26221 0 HF

TUMOR, LUNG

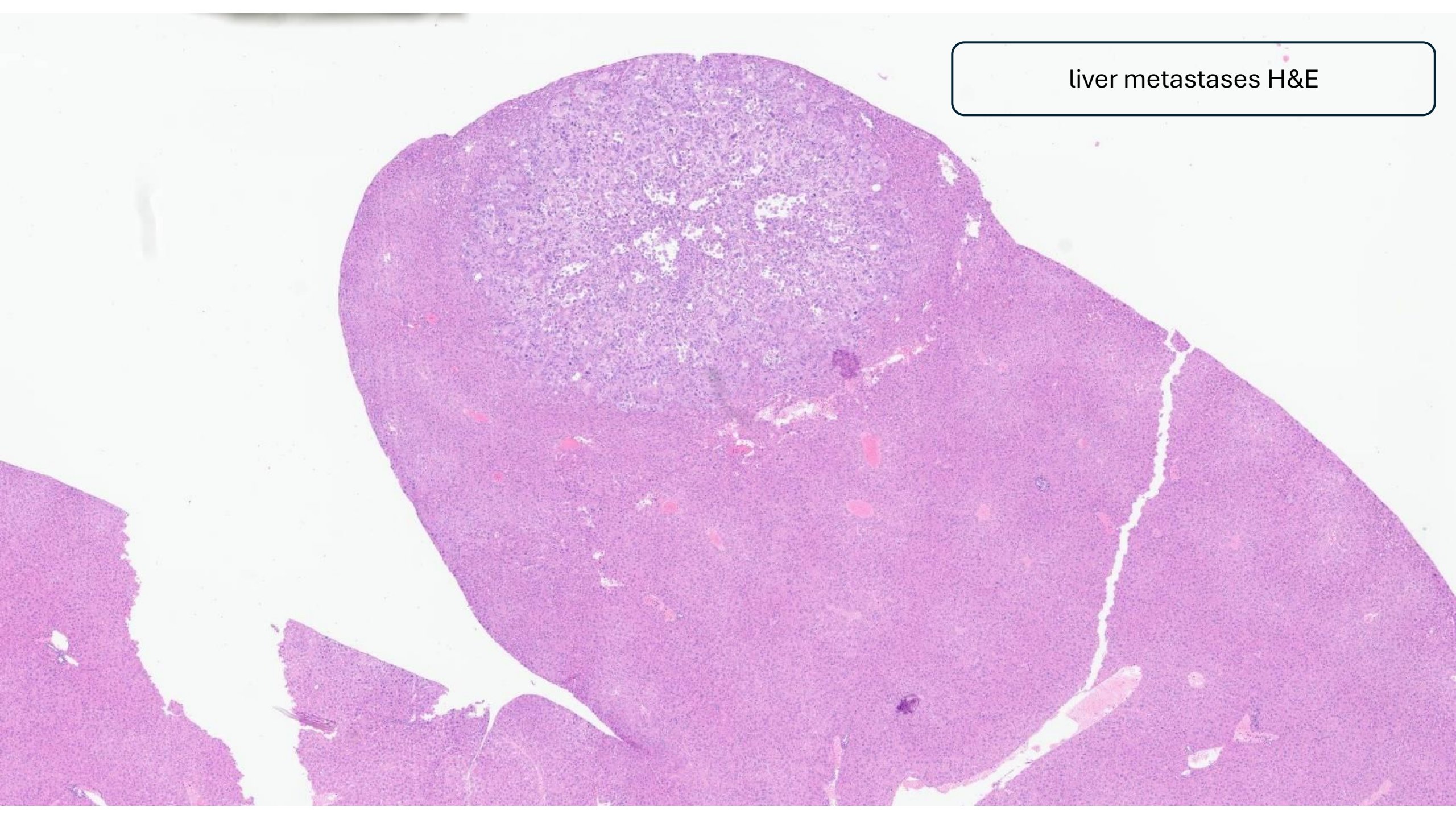
NP14 26221 1 PBS
NP14 26221 3 PBS
NP14 25284 2 PBS
NP14 26223 1 PBS
NP14 26482 1 PBS

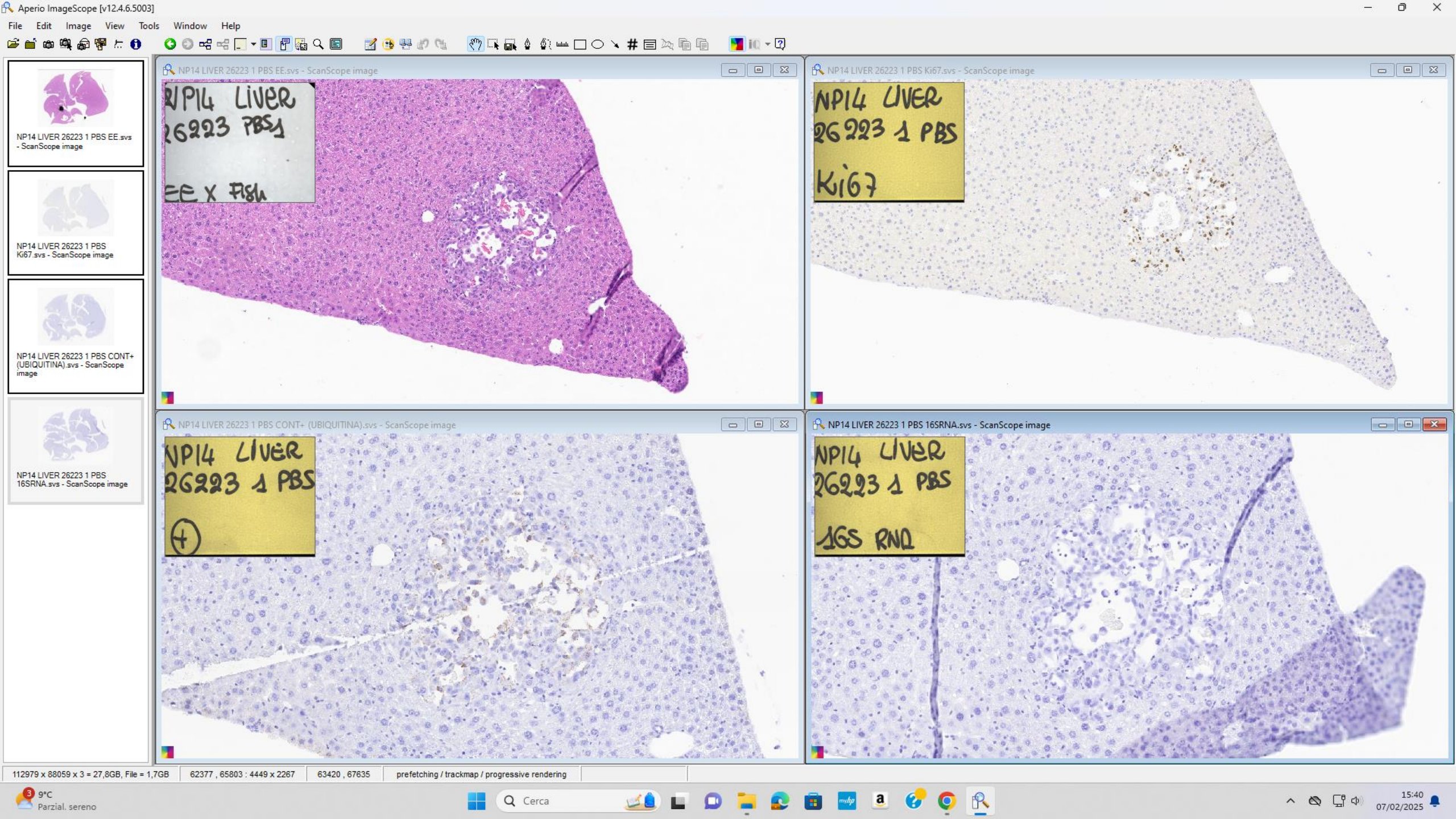
NP14 26221 2 SHIG
NP14 26485 0 SHIG
NP14 26485 1 SHIG
NP14 26485 2 SHIG
NP14 26223 0 SHIG

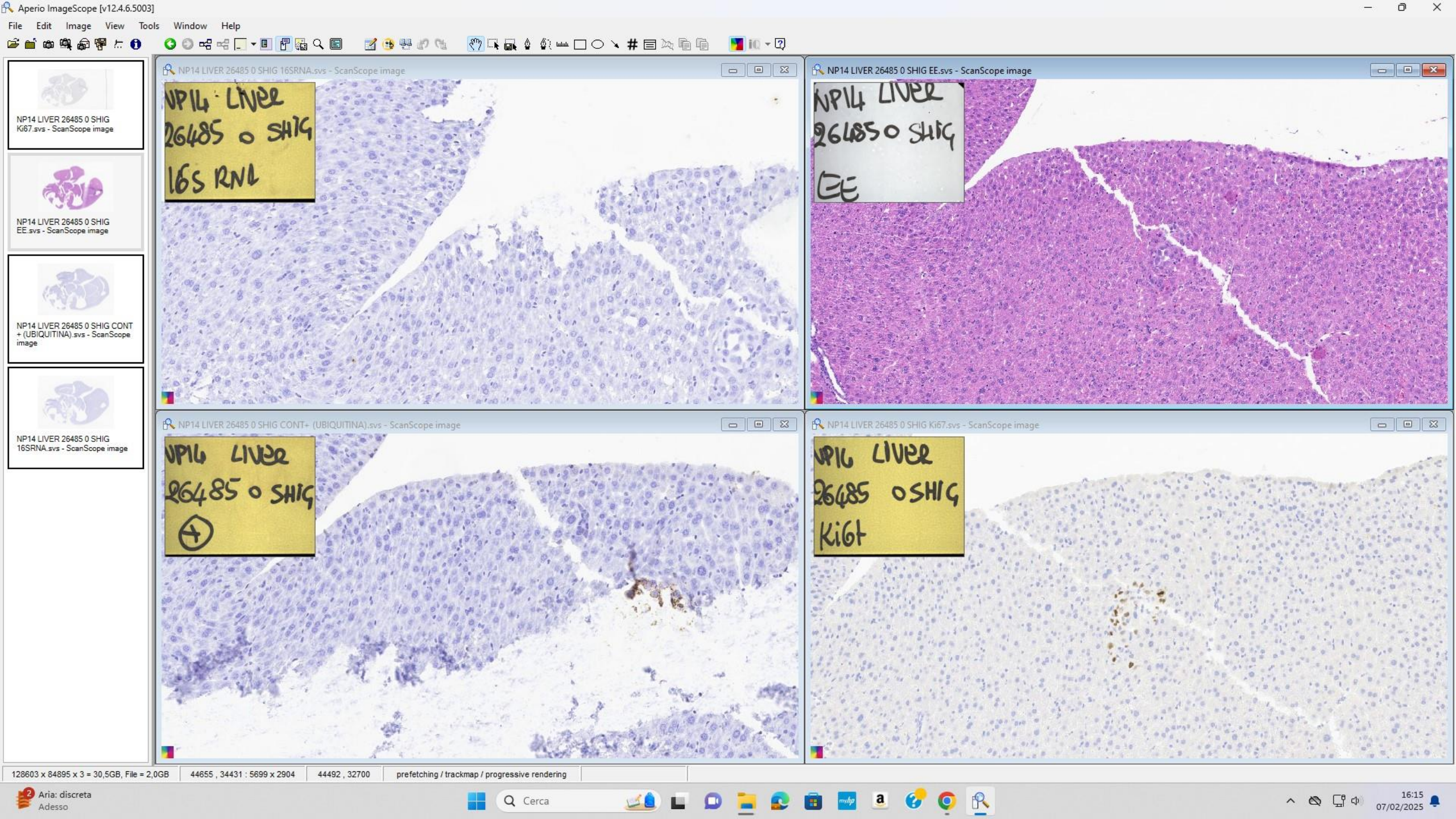
3

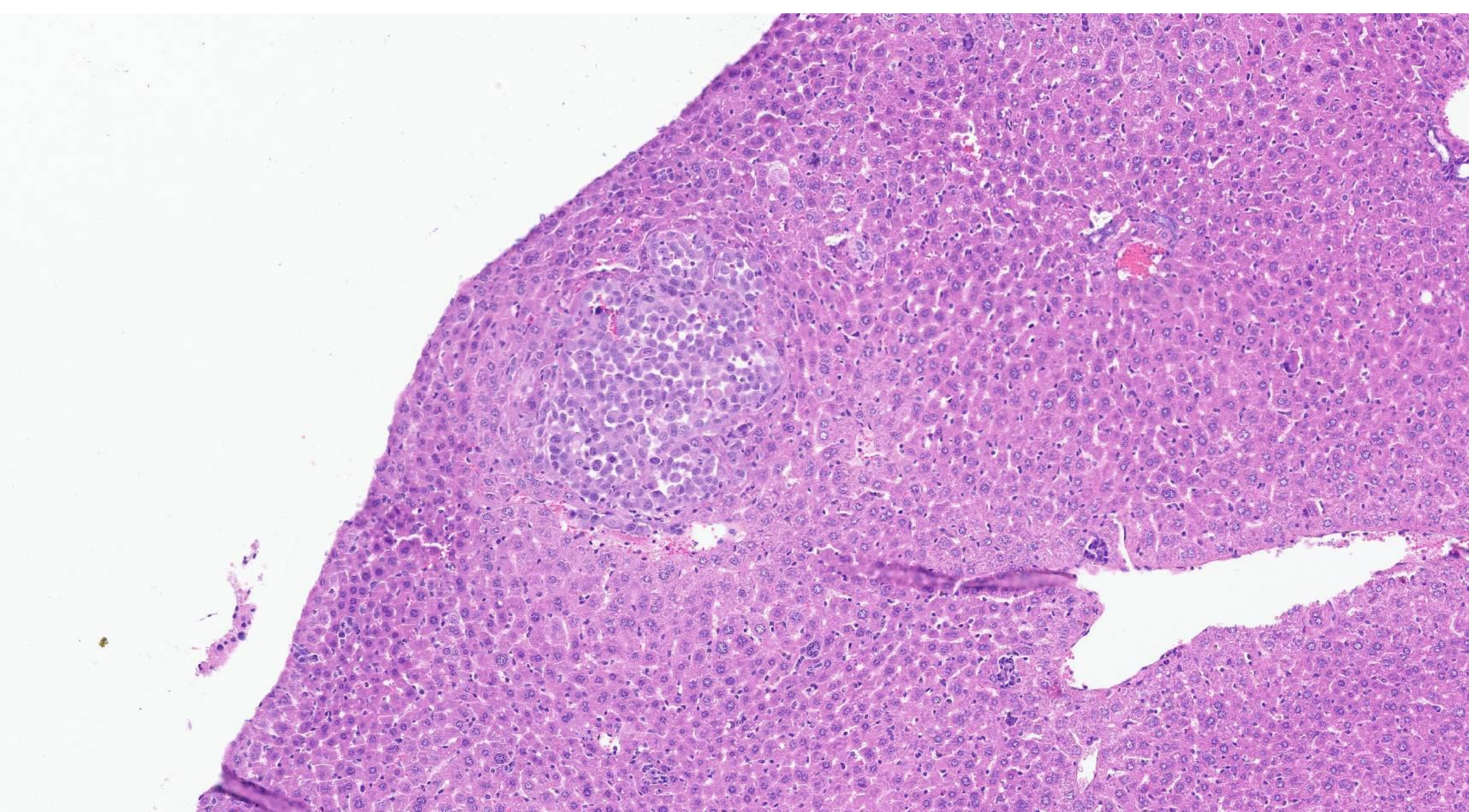
NO LUNG

liver metastases H&E

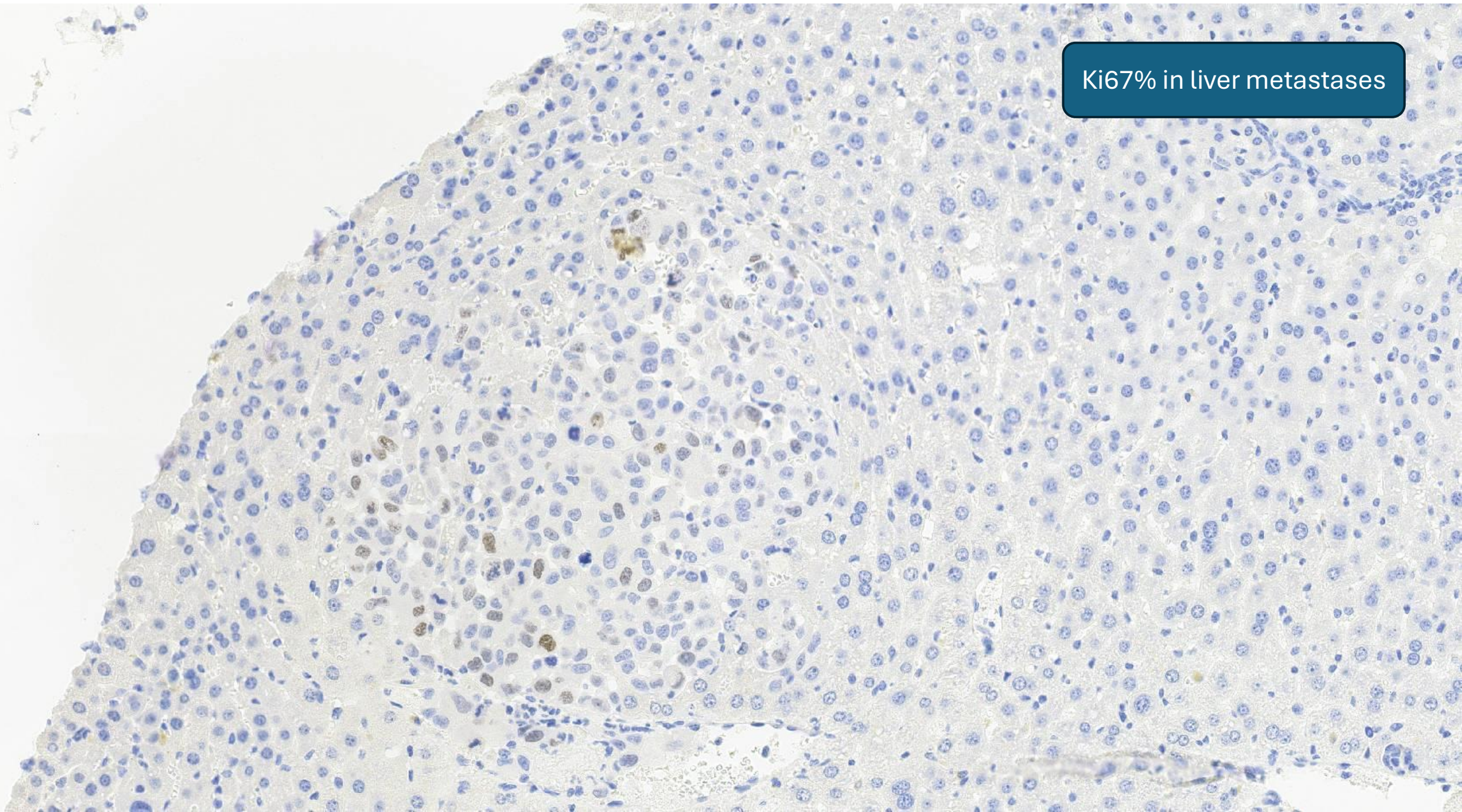




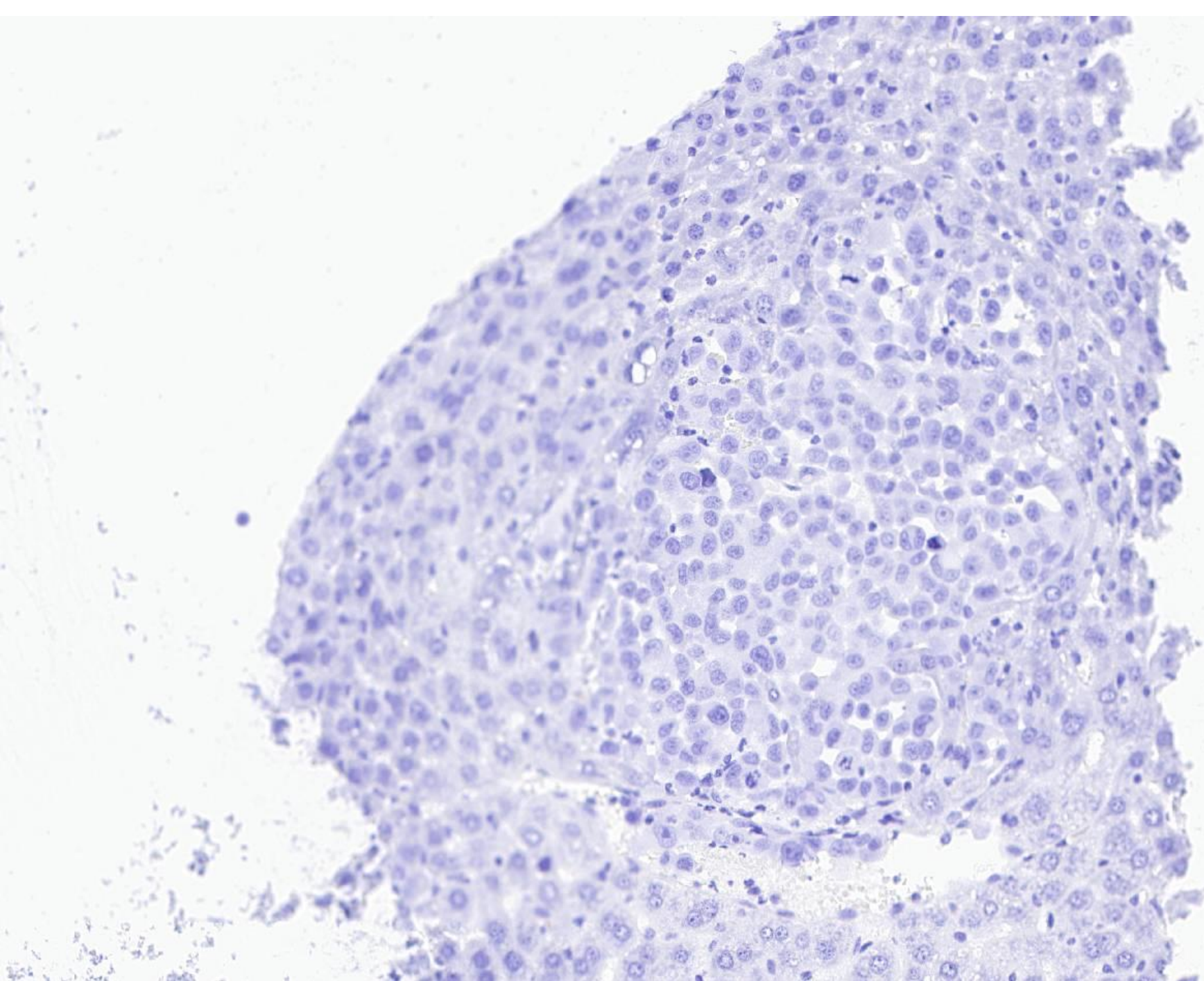


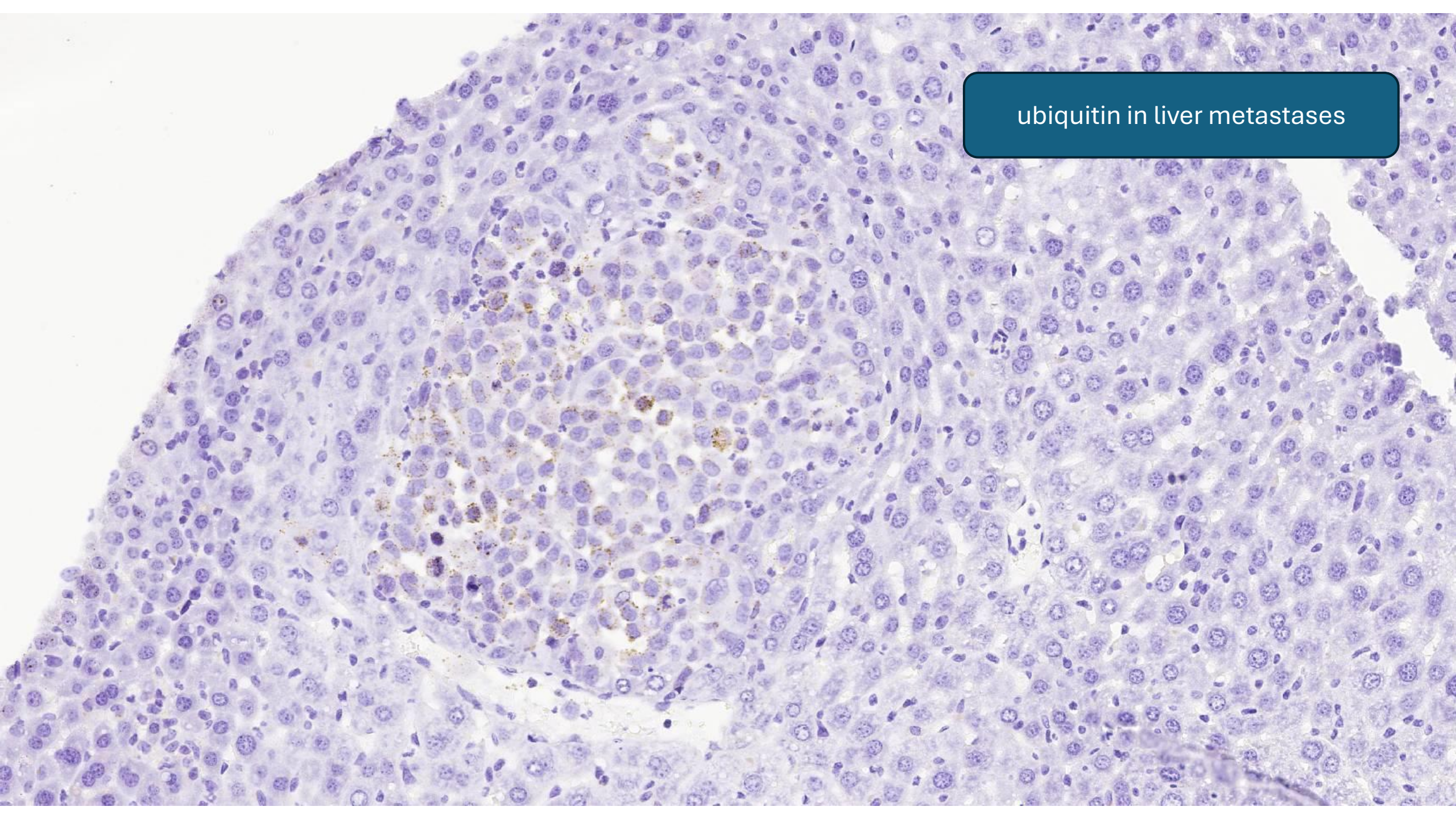


Ki67% in liver metastases



liver metastases, rRNA 16S

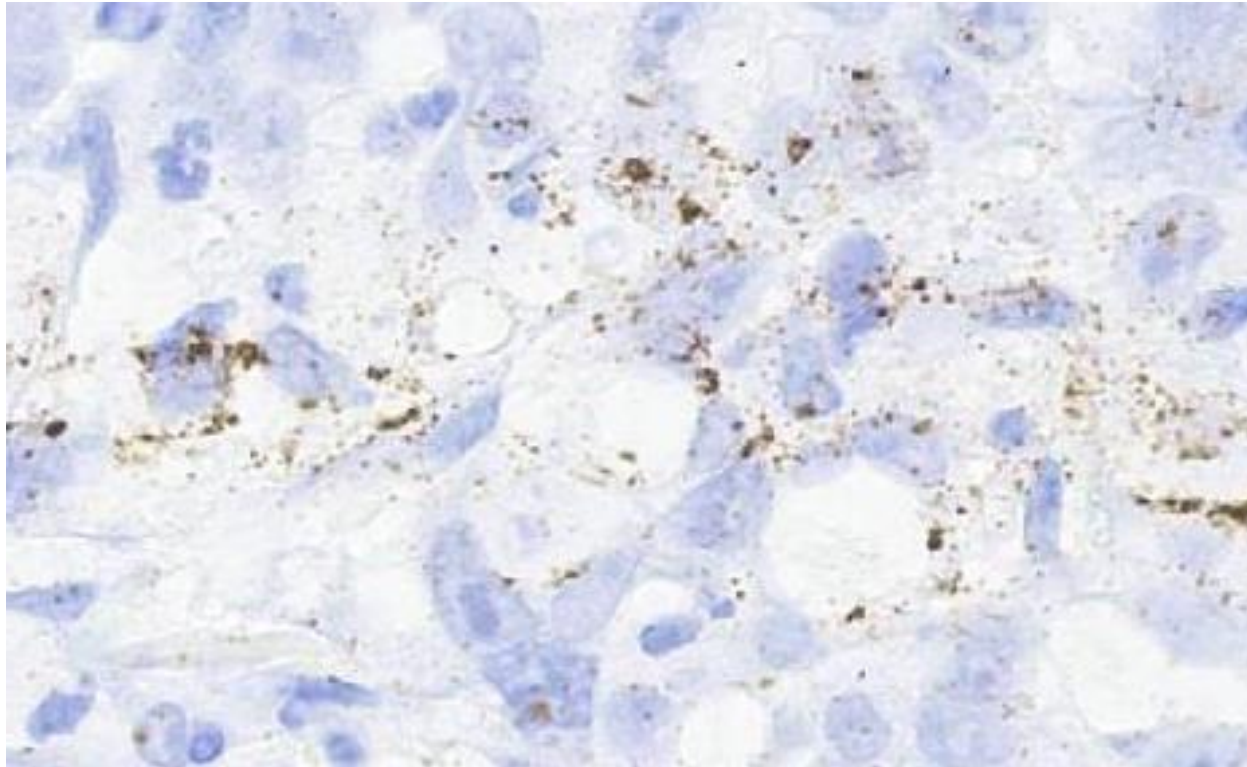




A histological section of liver tissue stained with hematoxylin and eosin (H&E). The image shows a dense population of cells with prominent, dark purple nuclei. The tissue architecture is somewhat disorganized, with many small, irregular clusters of cells interspersed among the larger, more uniform hepatocytes. These clusters represent metastatic deposits. The overall color is a mix of purple and pink, with some areas of lighter pink indicating less cellular density or possibly necrosis.

ubiquitin in liver metastases

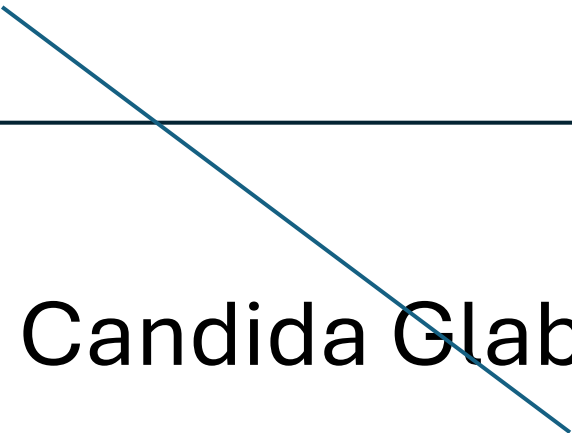
rRNA 16S primary tumor – mouse model



Bellinzona cohort

rRNA 16S has been observed in:

- 1) 42% (10/24) of Bellinzona cohort – mouse models
- 2) 54% (5/11) of primary tumors
- 3) 44% (4/9) of liver metastases
- 4) 20% (2/10) of pulmonary metastases



Candida Glabrata

Shighella Sonnei

12/02/25, 09:05

controlli pos/Nb (contiene
batteri)

NP14 26221 2 SHIG
NP14 26485 0 SHIG
NP14 26485 1 SHIG
NP14 26485 2 SHIG
NP14 26223 0 SHIG

Negative CTRL (non contiene batteri)

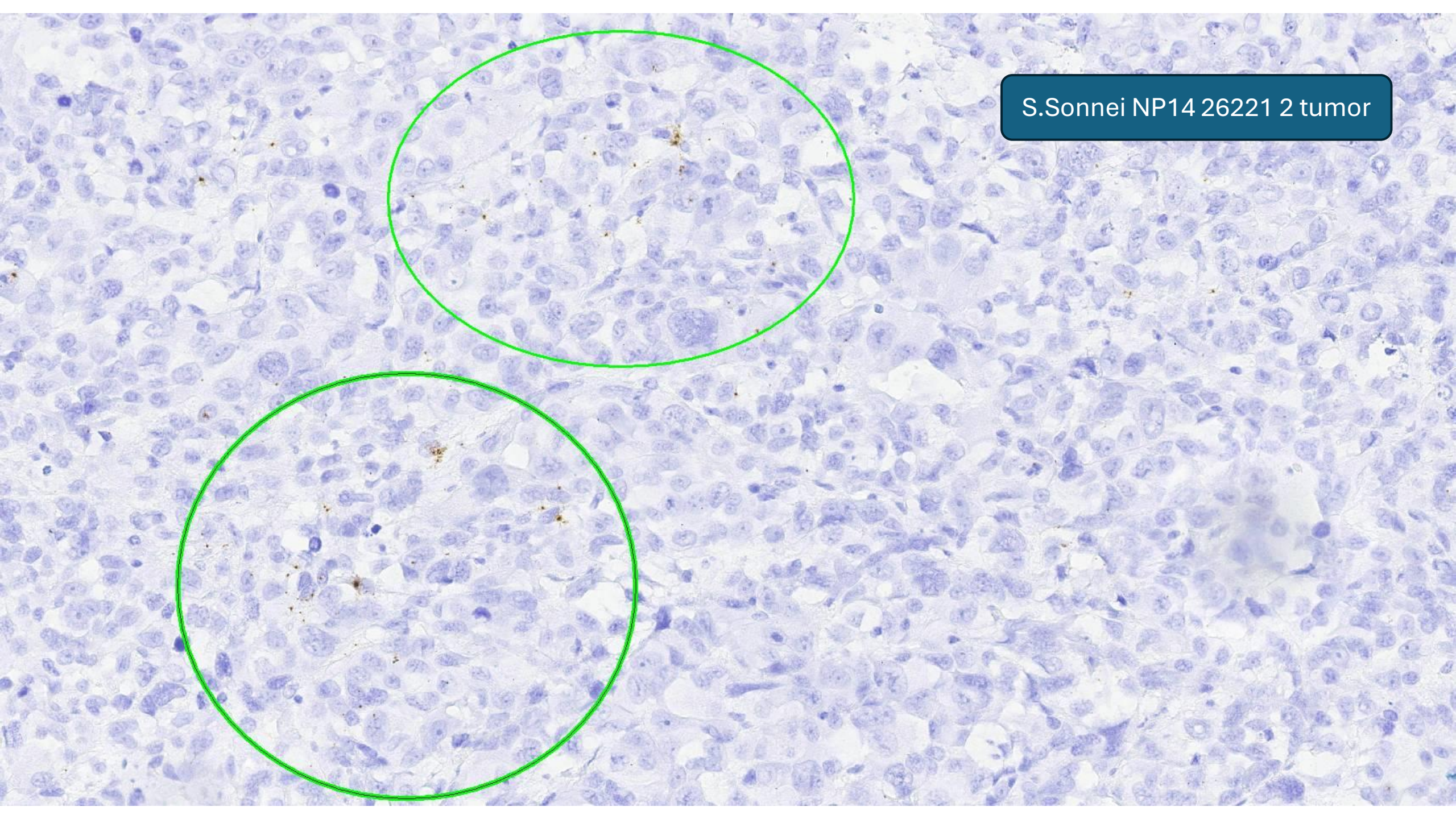
NP14 26221 1 PBS
NP14 26221 3 PBS
NP14 25284 2 PBS
NP14 26223 1 PBS
NP14 26482 1 PBS

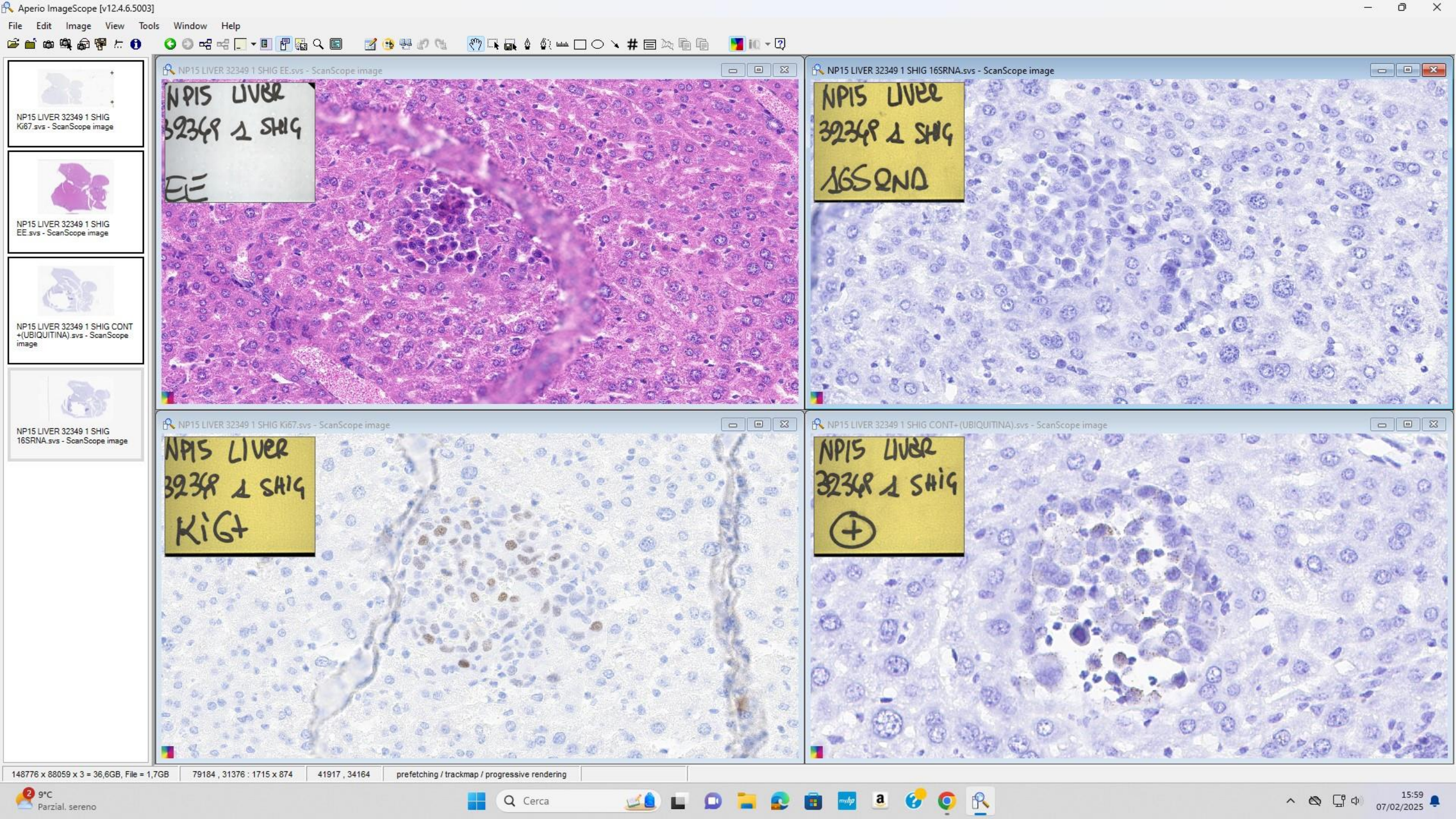
Shighella Sonnei

1/5, 20%

controls: all negative

S.Sonnei NP14 26221 2 tumor





NP15 LIVER 32349 1 SHIG Ki67.svs - ScanScope image

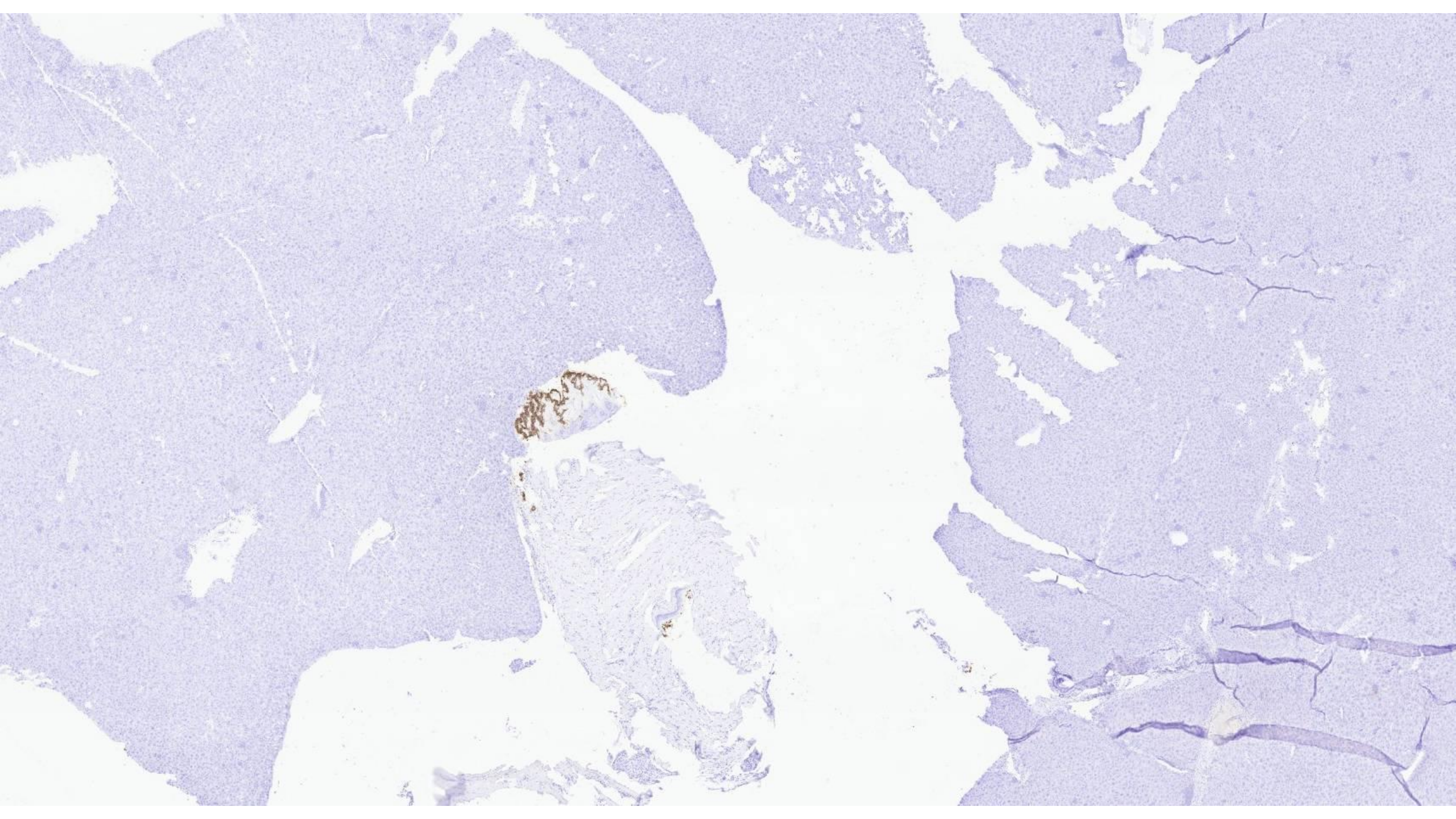
NP15 LIVER 32349 1 SHIG EE.svs - ScanScope image

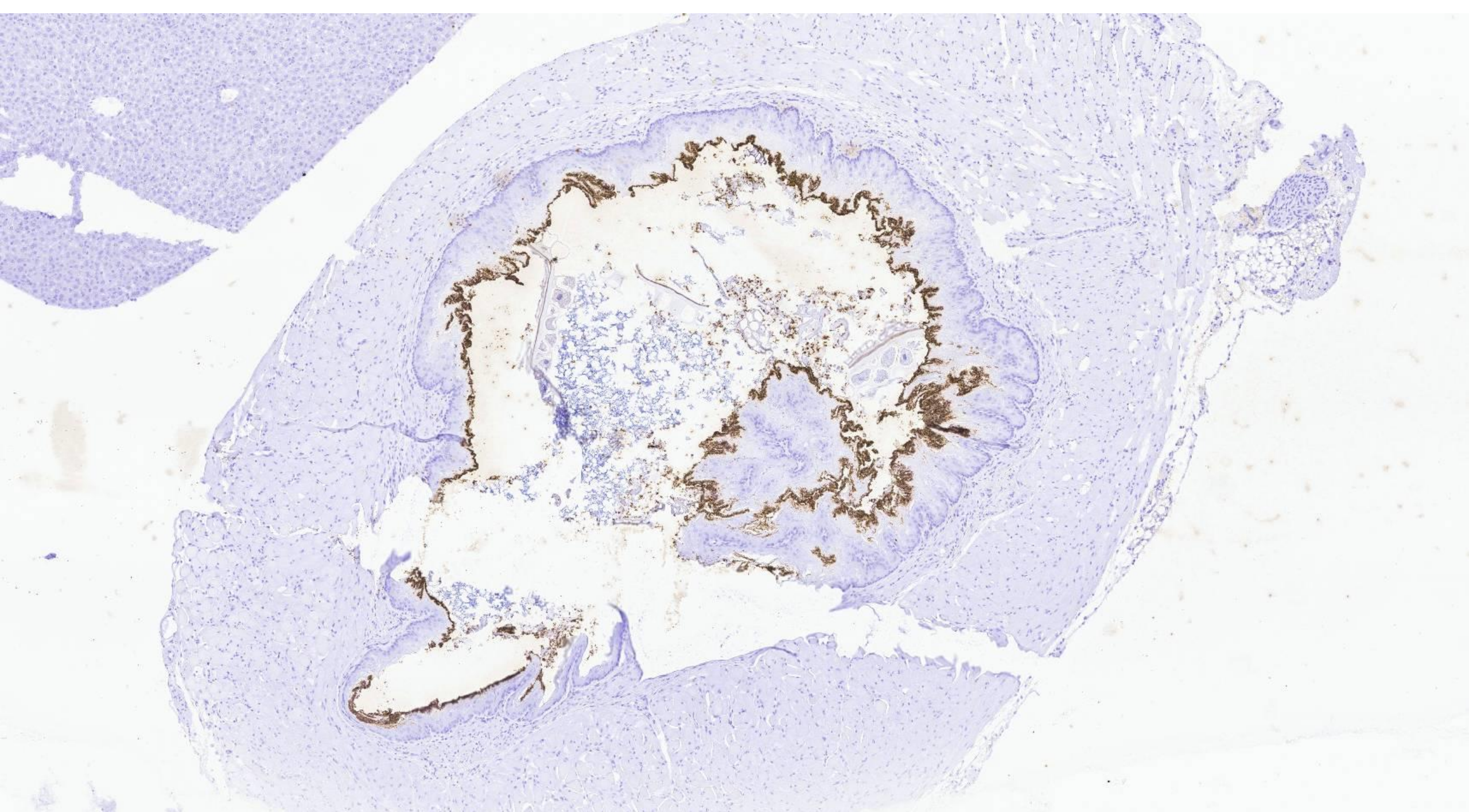
NP15 LIVER 32349 1 SHIG CONT+(UBIQUITINA).svs - ScanScope image

NP15 LIVER 32349 1 SHIG 16SRNA.svs - ScanScope image

NP15 LIVER 32349 1 SHIG Ki67.svs - ScanScope image

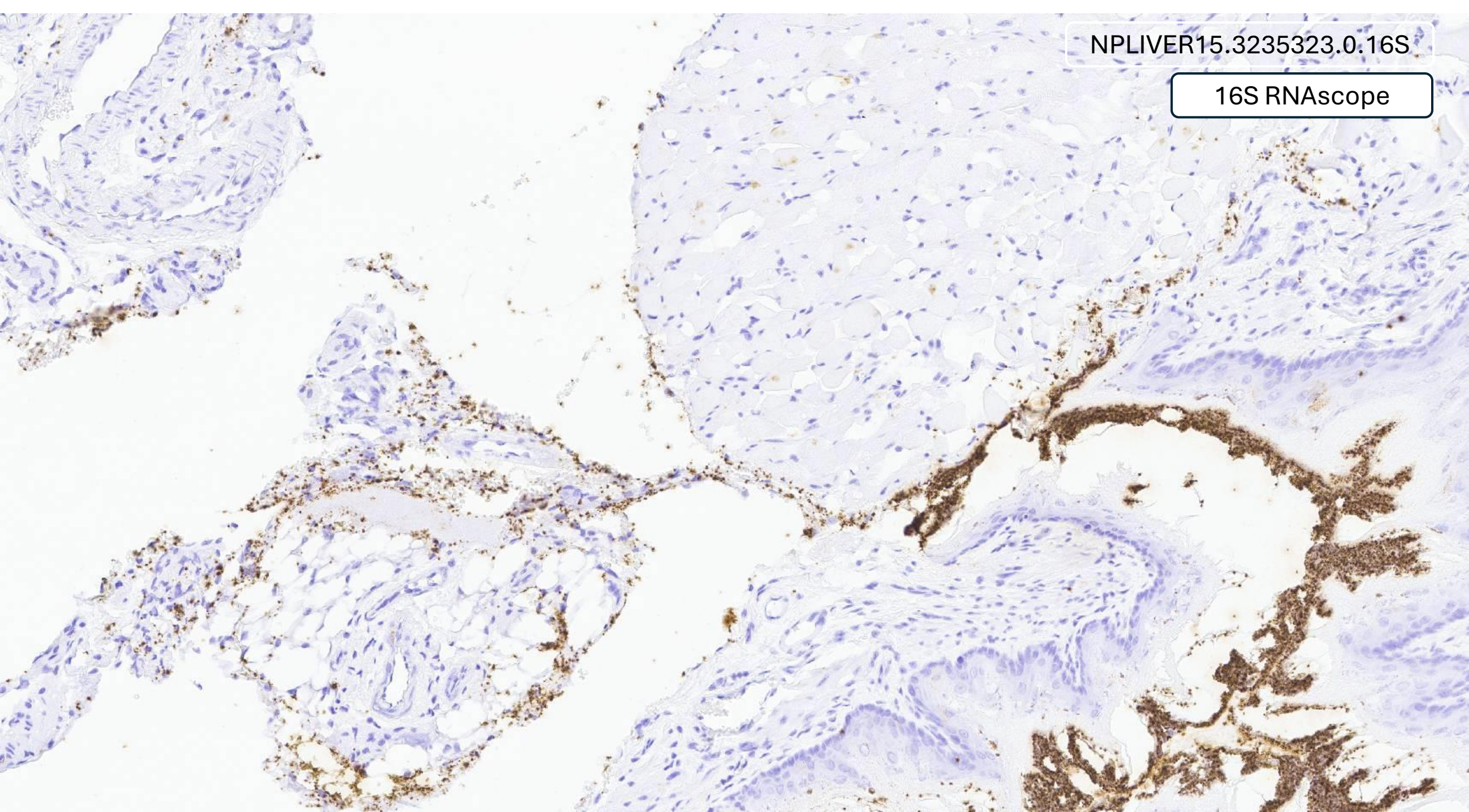
NP15 LIVER 32349 1 SHIG CONT+(UBIQUITINA).svs - ScanScope image



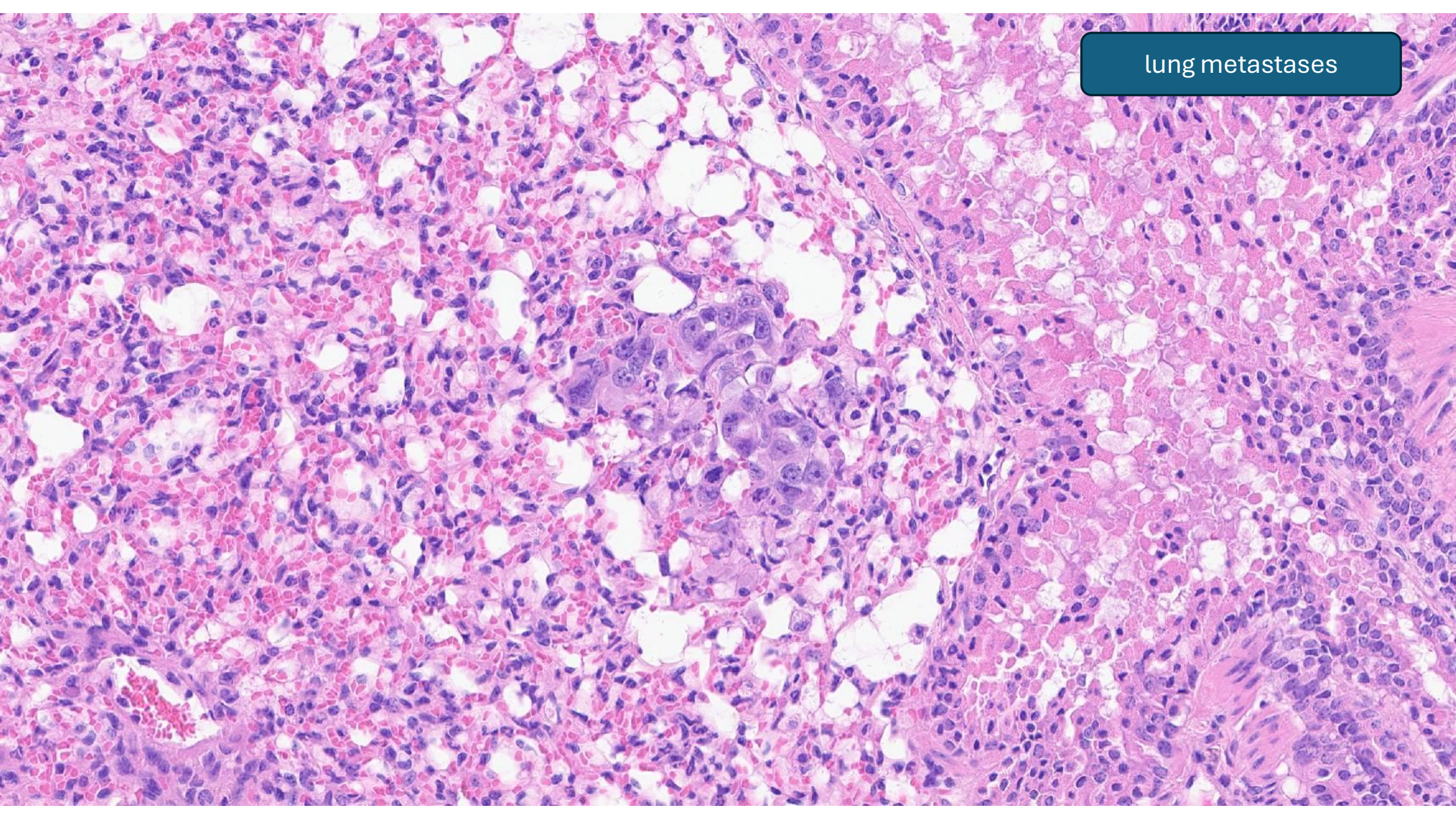


NPLIVER15.3235323.0.16S

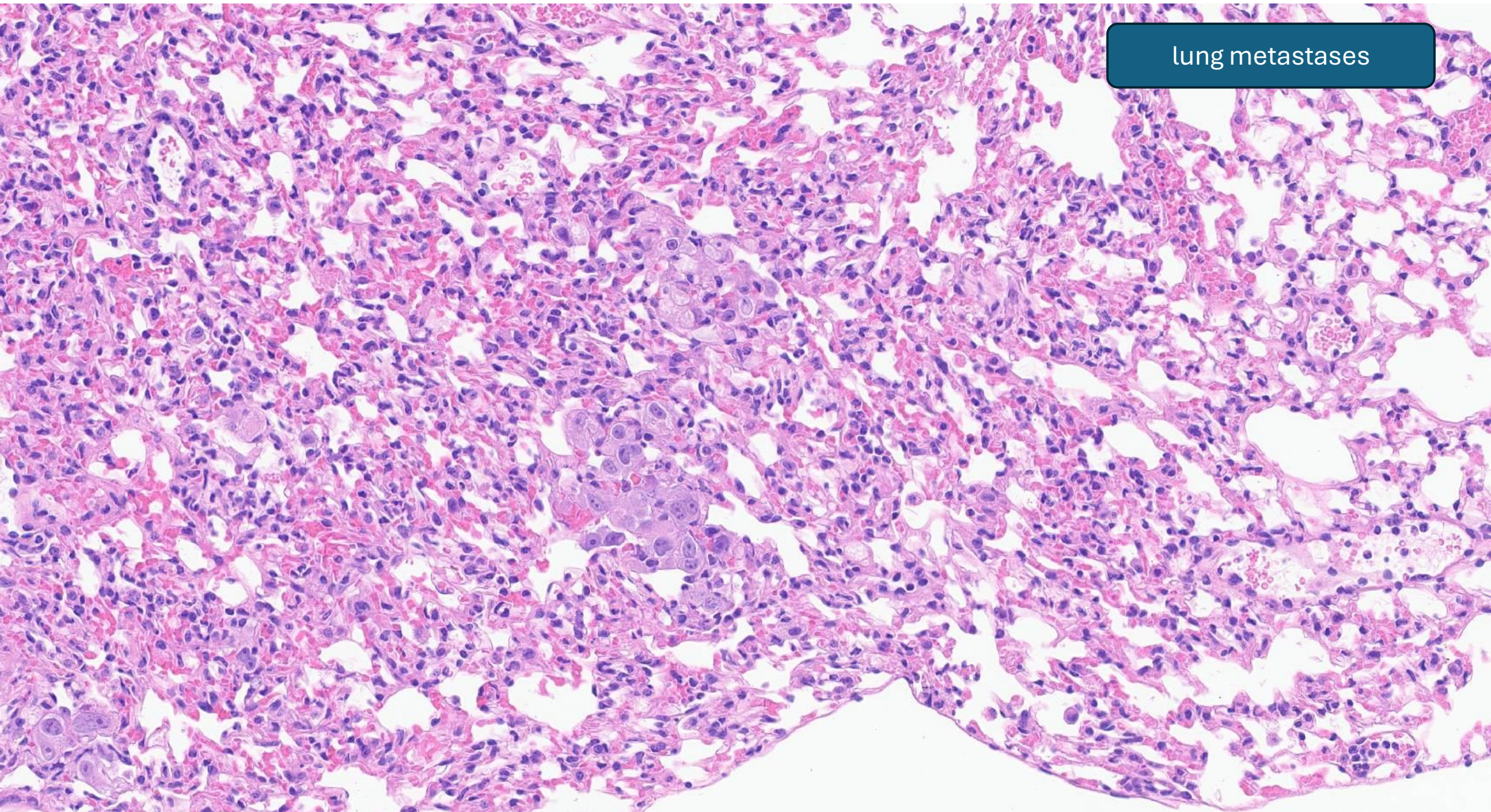
16S RNAscope



lung metastases



lung metastases



Actual results for discussion

- 1) rRNA 16S has been observed in 23% of castration resistant human prostate adenocarcinoma and in 11% of lymph-nodal metastases and 14% of visceral metastases
- 2) rRNA 16S has been observed in 42% of Bellinzona cohort – mouse models; 54% primary tumor, 44% liver metastases and 20% pulmonary metastases
- 3) S.Sonnei has been observed in 1/5 primary tumor (Bellinzona)

Future directions

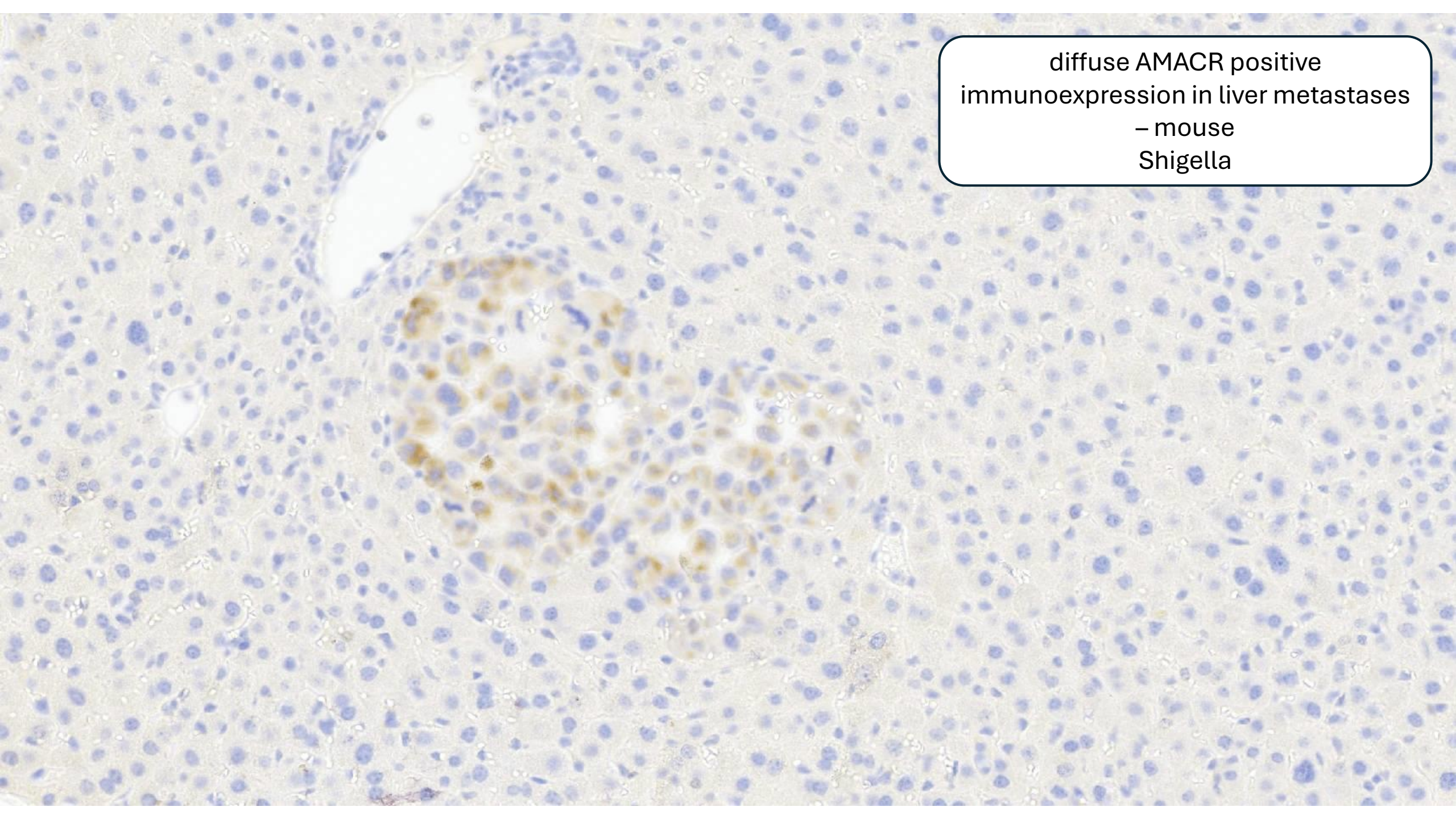
Shigella Sonnei to be completed on remaining cases

Candida Glabrata

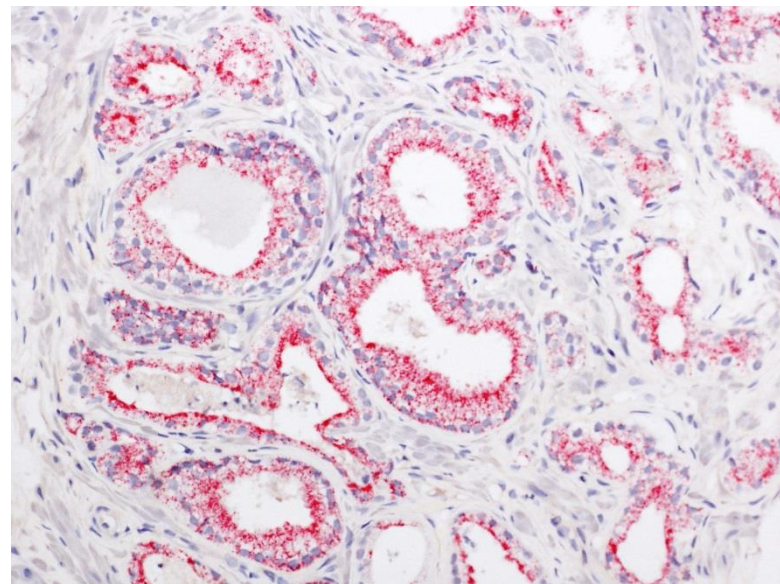
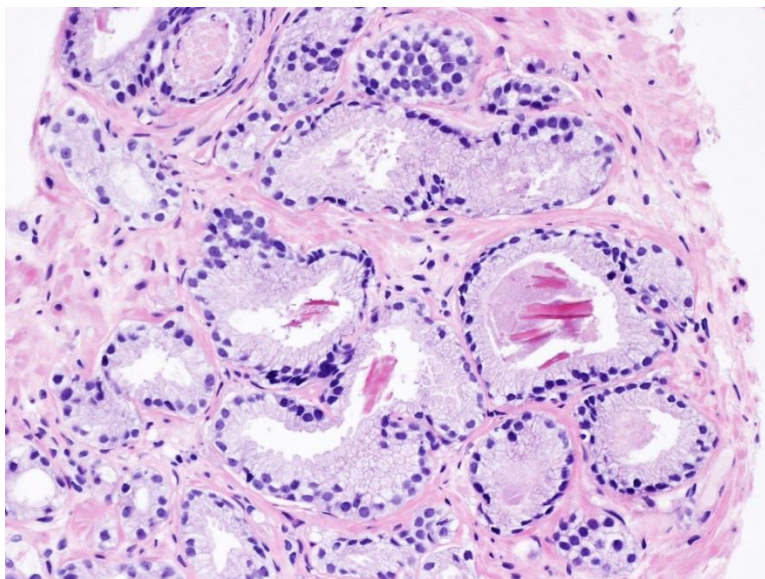
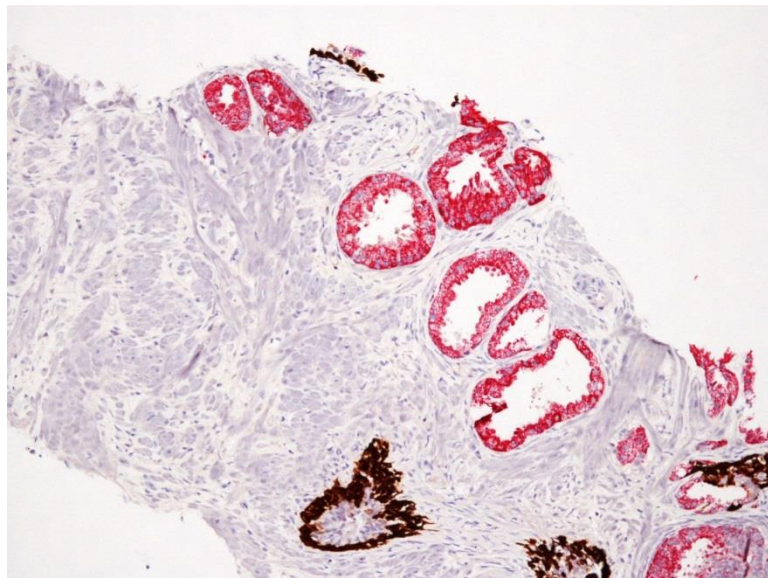
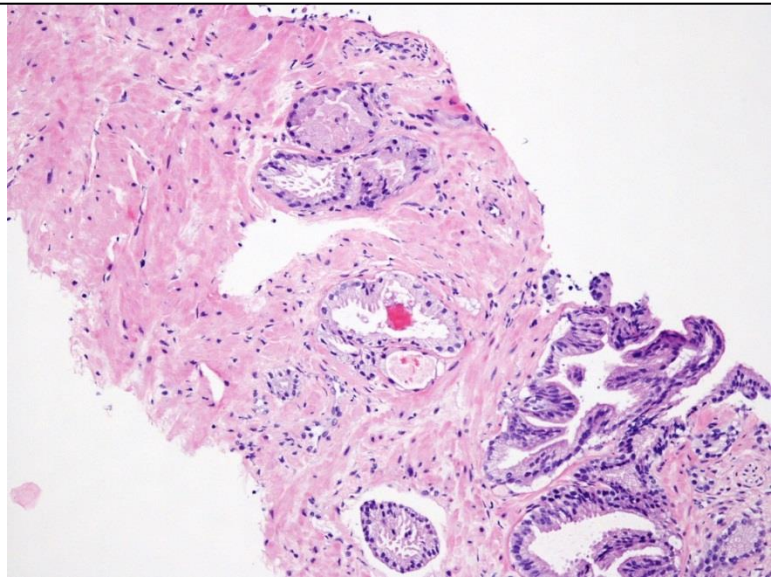
Additional find: AMACR immunoexpression in PSMA negative prostate adenocarcinoma

Tissue from bone metastases to be tested

diffuse AMACR positive
immunoexpression in liver metastases
– mouse
Shigella



AMACR and prostate carcinoma 2001



Meeting PRIN2022
March 28th, 2025, 09.30-13.00
IOR, Via F. Chiesa 5, Bellinzona
Meeting Room 1 North West

Draft

PRIN2022: Targeting the intratumoral microbiota to reverse therapeutic resistance in lethal prostate cancers

Meeting objective: To provide an update on the current state of research and share the latest findings on the role of microbiota in cancer, with a particular focus on data from three research units.

11.30 – 12.15

Session 3: Intratumoral Microbiota and Pathology: The UNIVR Experience

Updates from Research Unit 3

Speaker: Matteo Brunelli

Topic: “RNAscope and ISH Technology and Intratumoral Microbiota”