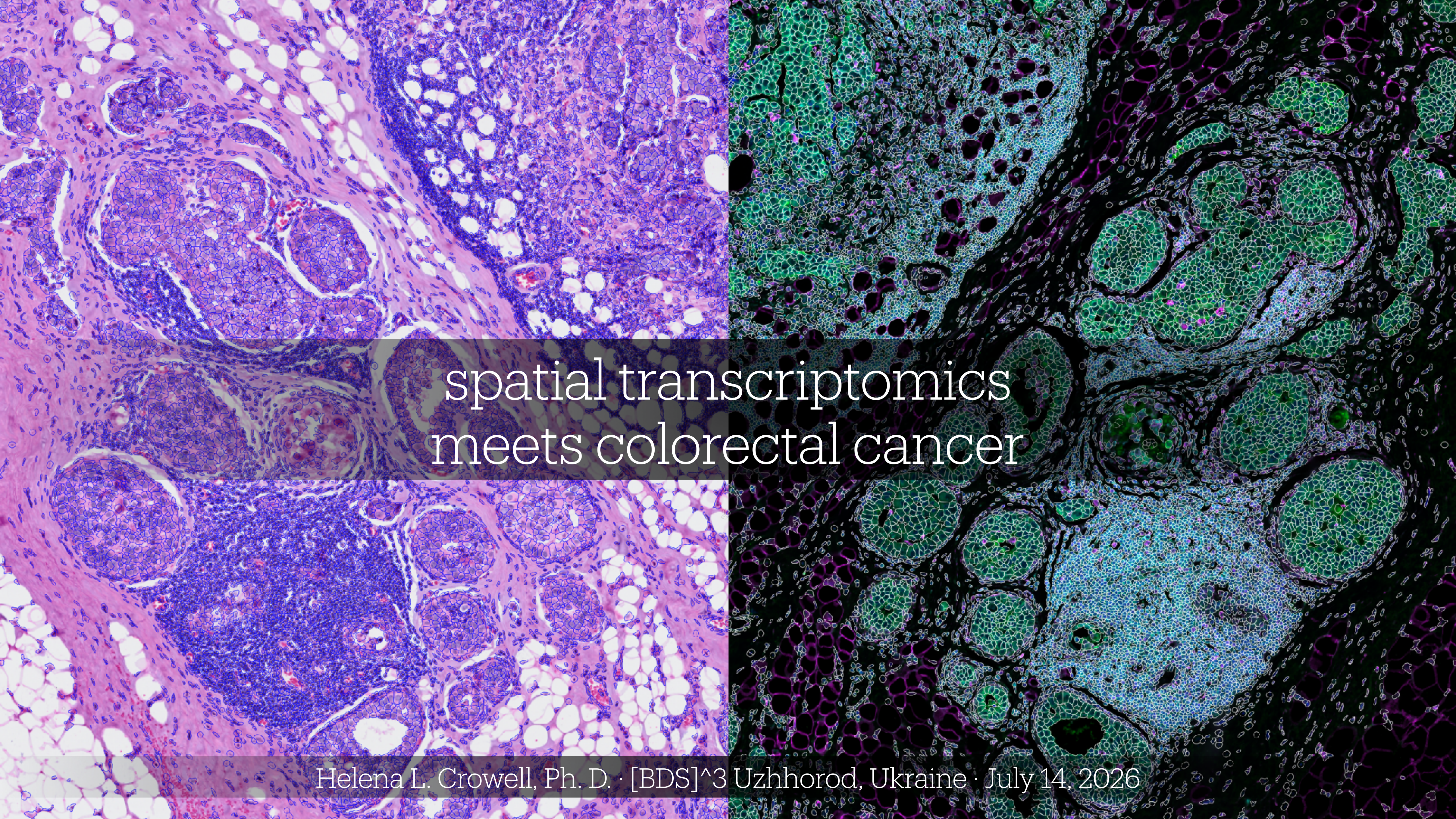
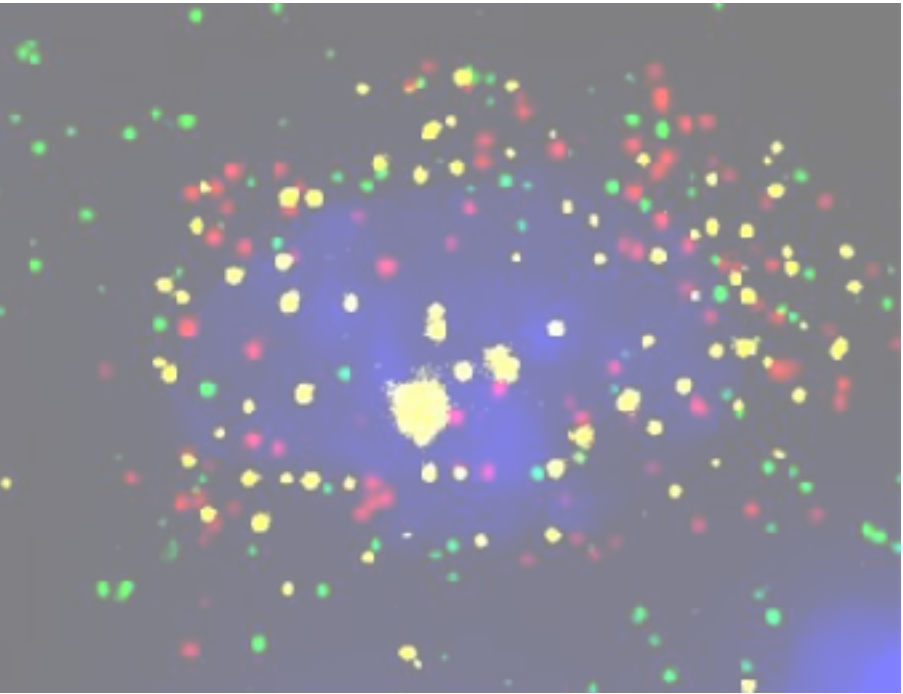
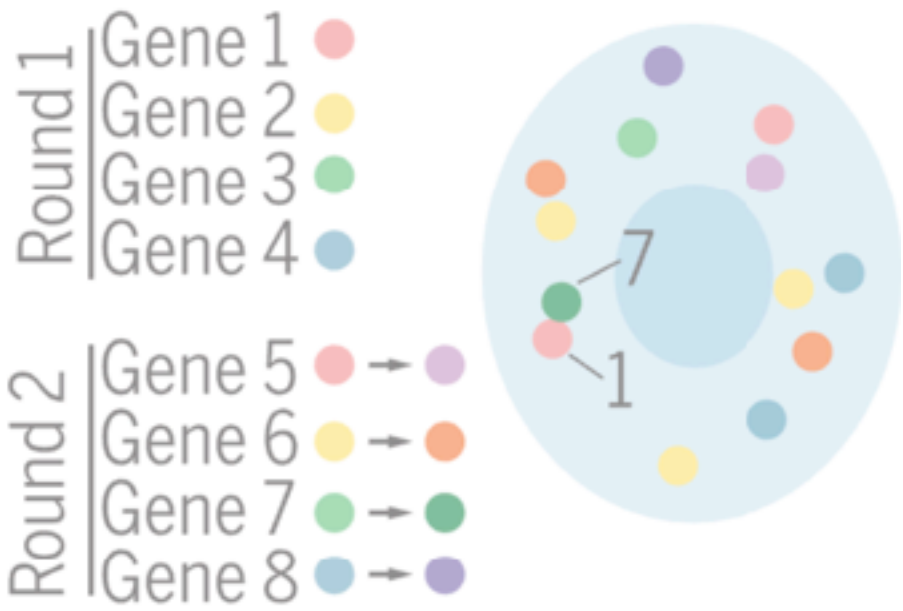
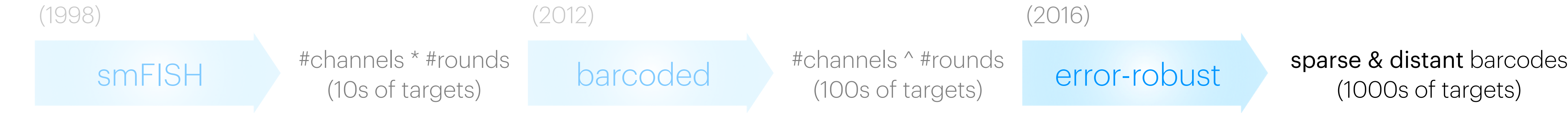




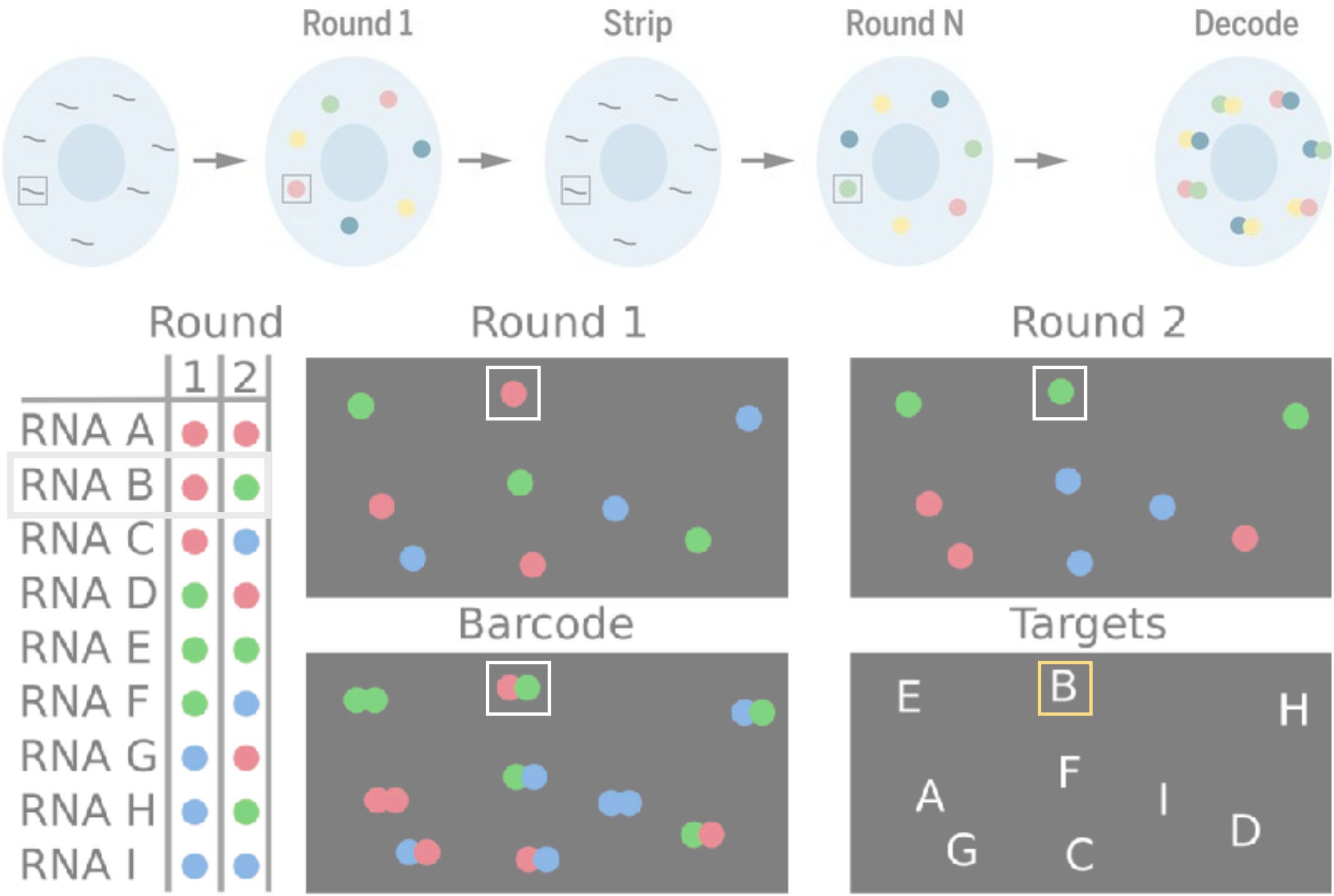
spatial transcriptomics meets colorectal cancer

Helena L. Crowell, Ph. D. · [BDS]³ Uzhhorod, Ukraine · July 14, 2026

error-robust barcoded FISH

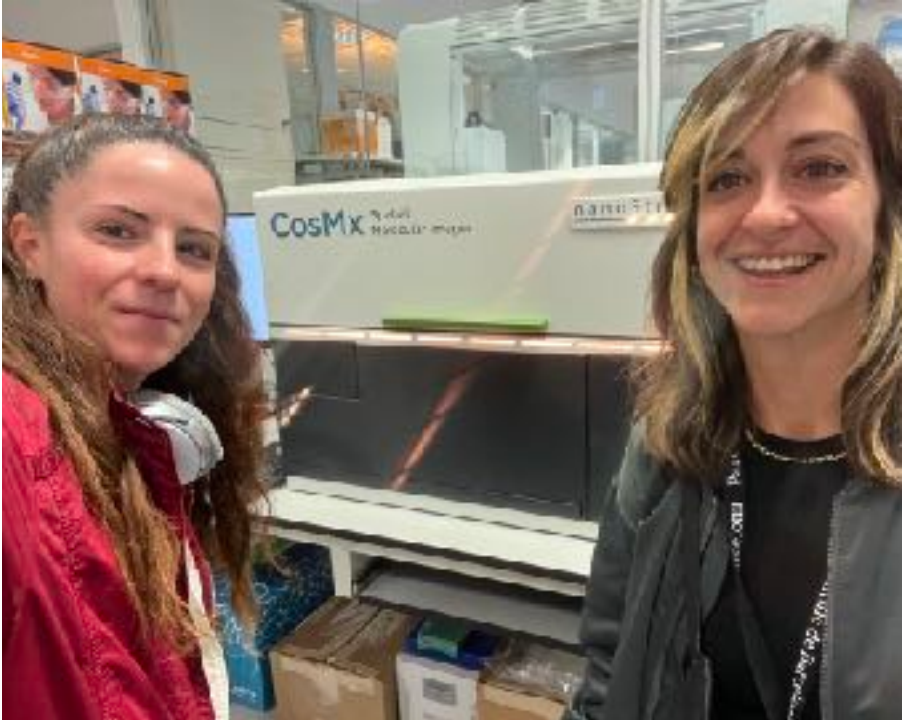


gene 1 gene 2 gene 3 DNA



	Round 1				Round 2			
Bit	1	2	3	4	5	6	7	8
Gene 1	1	0	0	0	0	0	1	0
Gene 2	0	0	1	0	1	0	0	0
Gene 3	1	0	0	0	0	1	0	0
Gene n	0	0	0	1	0	0	0	1

CosMx (Bruker)
MERSCOPE (Vizgen)
Xenium (10x Genomics)



layer controls

opacity: 0.00

blending: translucent

label: 1

brush size: 10

color mode: auto

contour: 0

n edit dim: 2

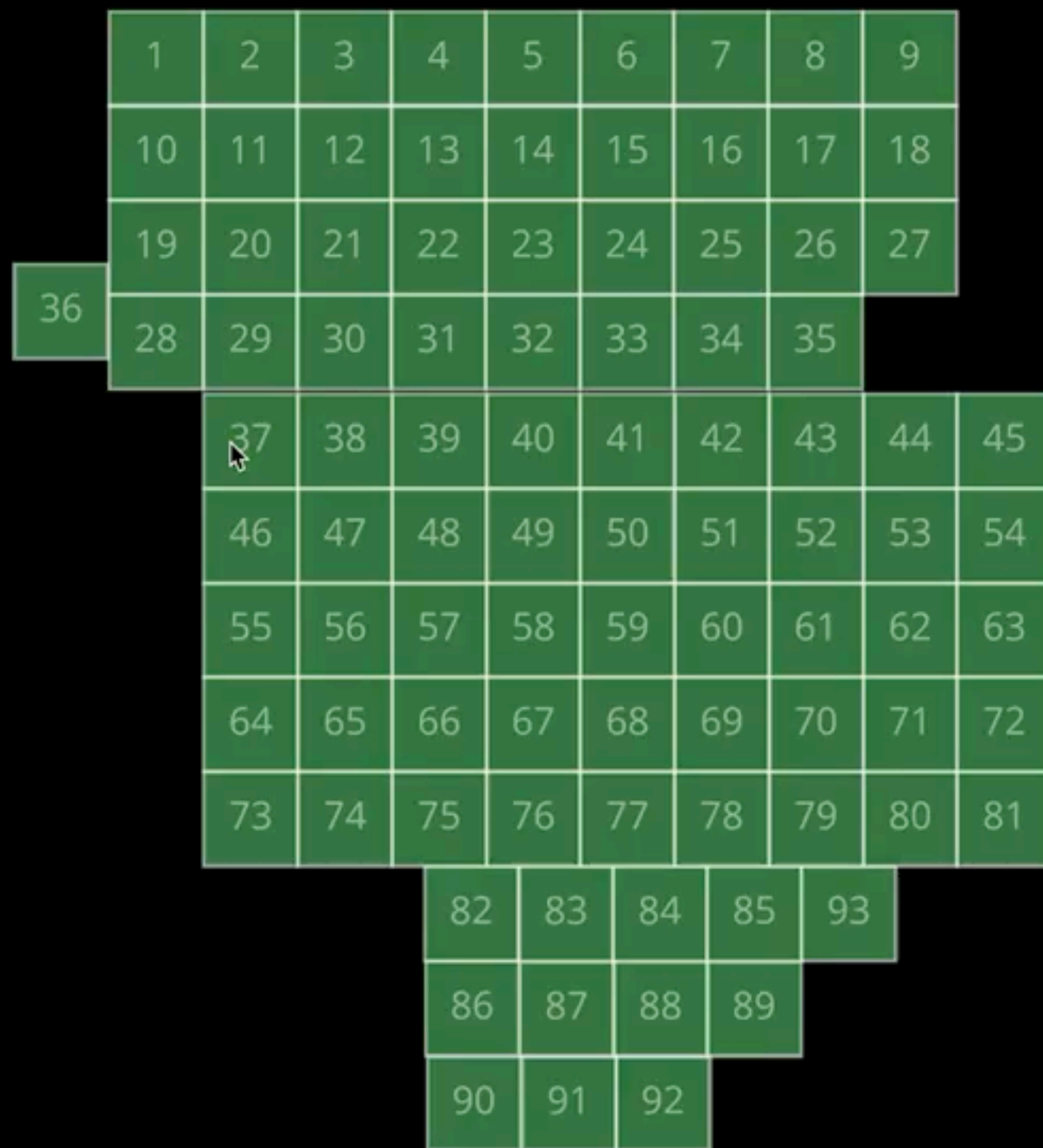
contiguous: ☒

preserve labels: ☐

show selected: ☐

layer list

- FOV labels
- Segmentation
- IGHA1
- CD19
- Metadata
- CD45
- DAPI



Morphology Images

channel: B

colormap: yellow

Add layer

RNA Transcripts

target: IGHA1

color: yellow

Add layer

Color Cells

column:

Stitch Images

To create napari-ready zarr files, select raw data folder:

Browse...

No folder selected.

Choose Output Folder

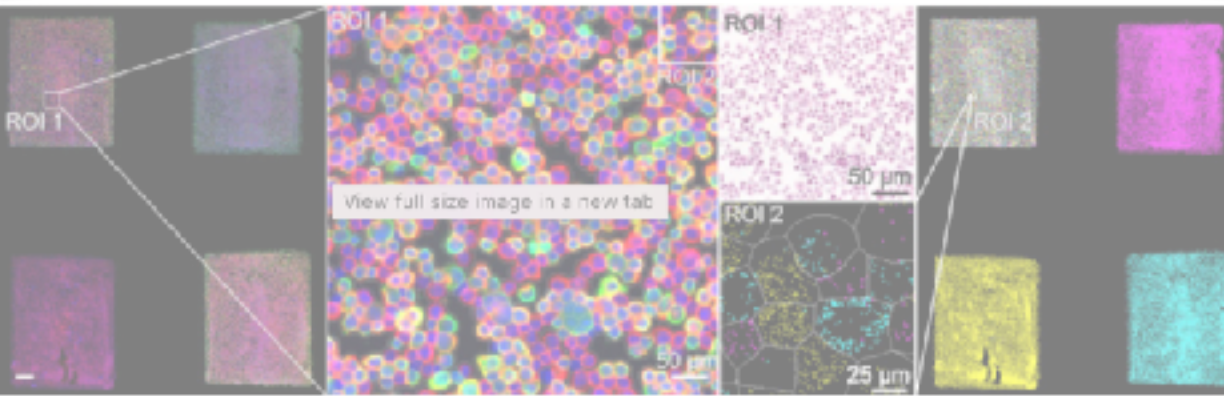
Stitch

```
viewer.layers["DAPI"].contrast_limits = [50, 200]
viewer.layers["CD45"].contrast_limits = [220, 580]
viewer.layers["PanCK"].contrast_limits = [250, 1000]
viewer.layers["PanMem"].contrast_limits = [150, 700]
```


real-life research: colorectal cancer

STAMP: Single-cell transcriptomics analysis and multimodal profiling through imaging

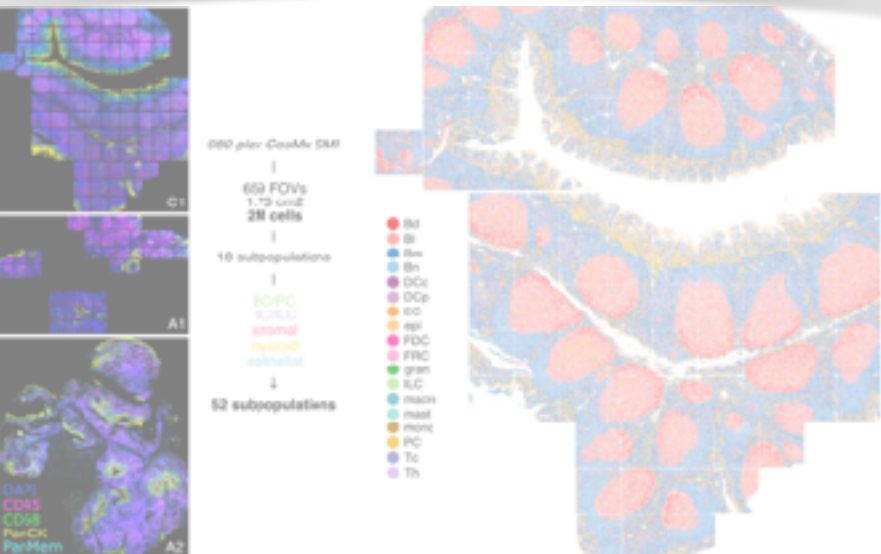
Emanuele Pitino^{1,14} · Anna Pascual-Reguant^{1,2,14} ·
Felipe Segato-Dezem^{3,4,14} · ... · Jasmine T. Plummer^{3,4,9,12} ·
Halger Heyn^{1,2,13} · Luciano G. Martelotto^{5,6,15} ... Show more



imaging instead
of sequencing

A transcriptional map of human tonsil architecture: beyond the sum of (single cell) parts

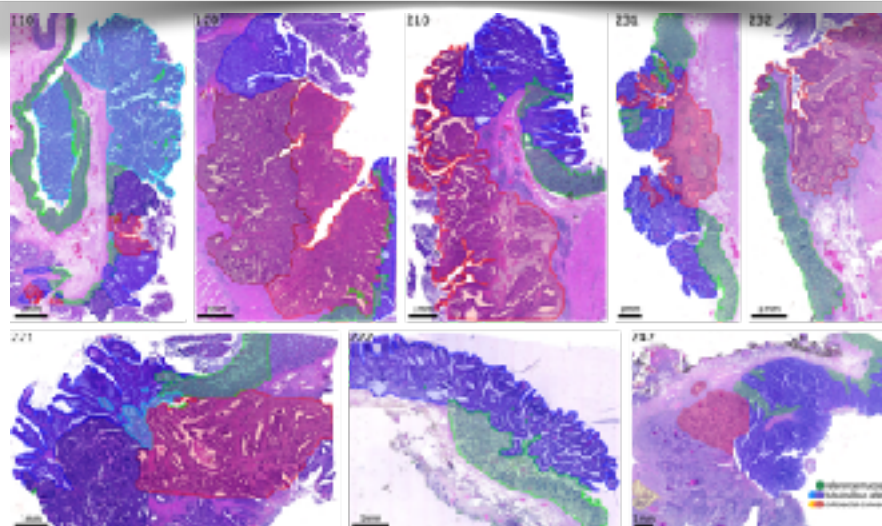
Helena L. Crowell¹, Laura Lab-Cid², Gerard Frigola³, Samuel Gunc^{4,5}, Irene Ruano¹,
Patricia Lorden¹, Max Ruiz¹, Maria Kulis^{2,4}, José Ignacio Martín-Suero^{1,6,7,8},
Hoger Heyn^{1,7,8}, Elias Campo^{2,3,7} & Anna Pascual-Reguant^{1,7,8}



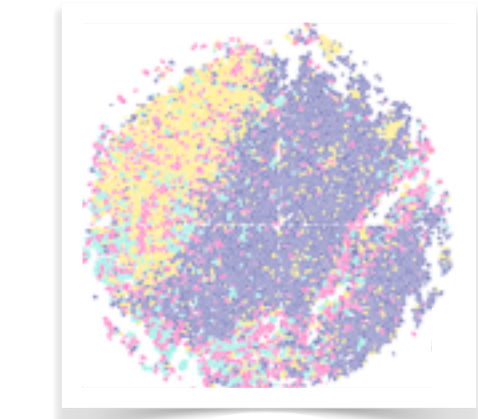
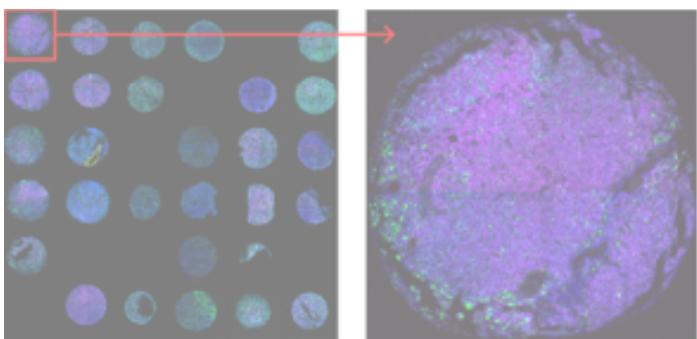
1k-plex CosMx on
human tonsil

Tracing colorectal malignancy transformation from cell to tissue scale

Helena L. Crowell¹, Irene Ruano¹, Zedong Hu², Youme Hong², Glin Corral³, Hubert Pessevaux²,
Ashley Hack⁴, Rachel Uhl⁴, Max Walter⁴, Megan Vandenberg⁴, Kim Young⁴, Dan McGuire⁴,
Evelyn Metzger⁴, Margaret L. Hoang², Joseph M. Sauter⁴, Sabine Tejpar²,
Anna Pascual-Reguant^{1,2,4} & Halger Heyn^{1,5,6,7}



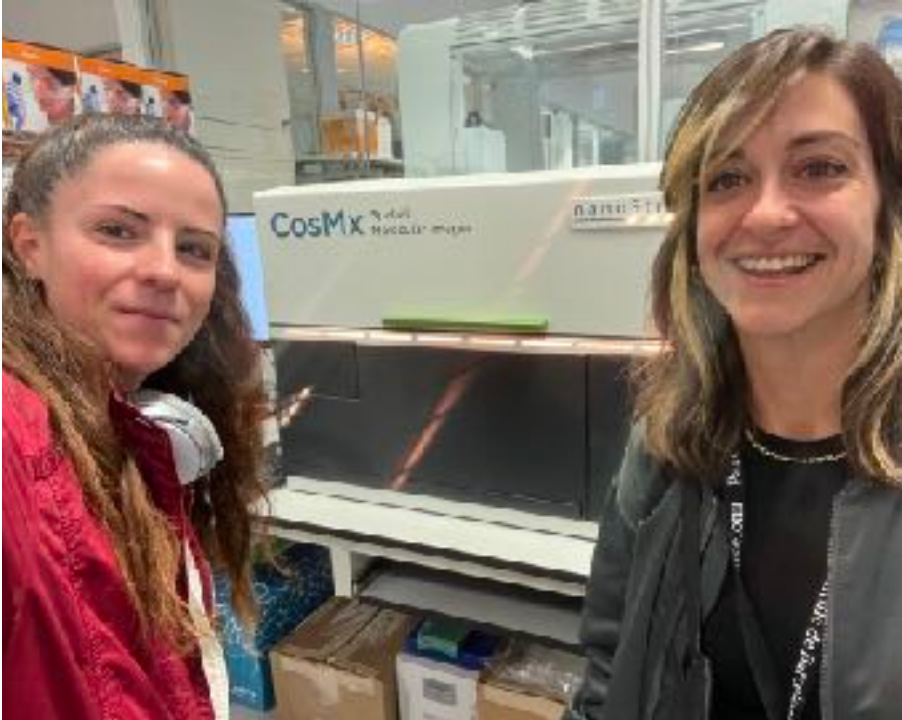
19k-plex CosMx on
colorectal cancer



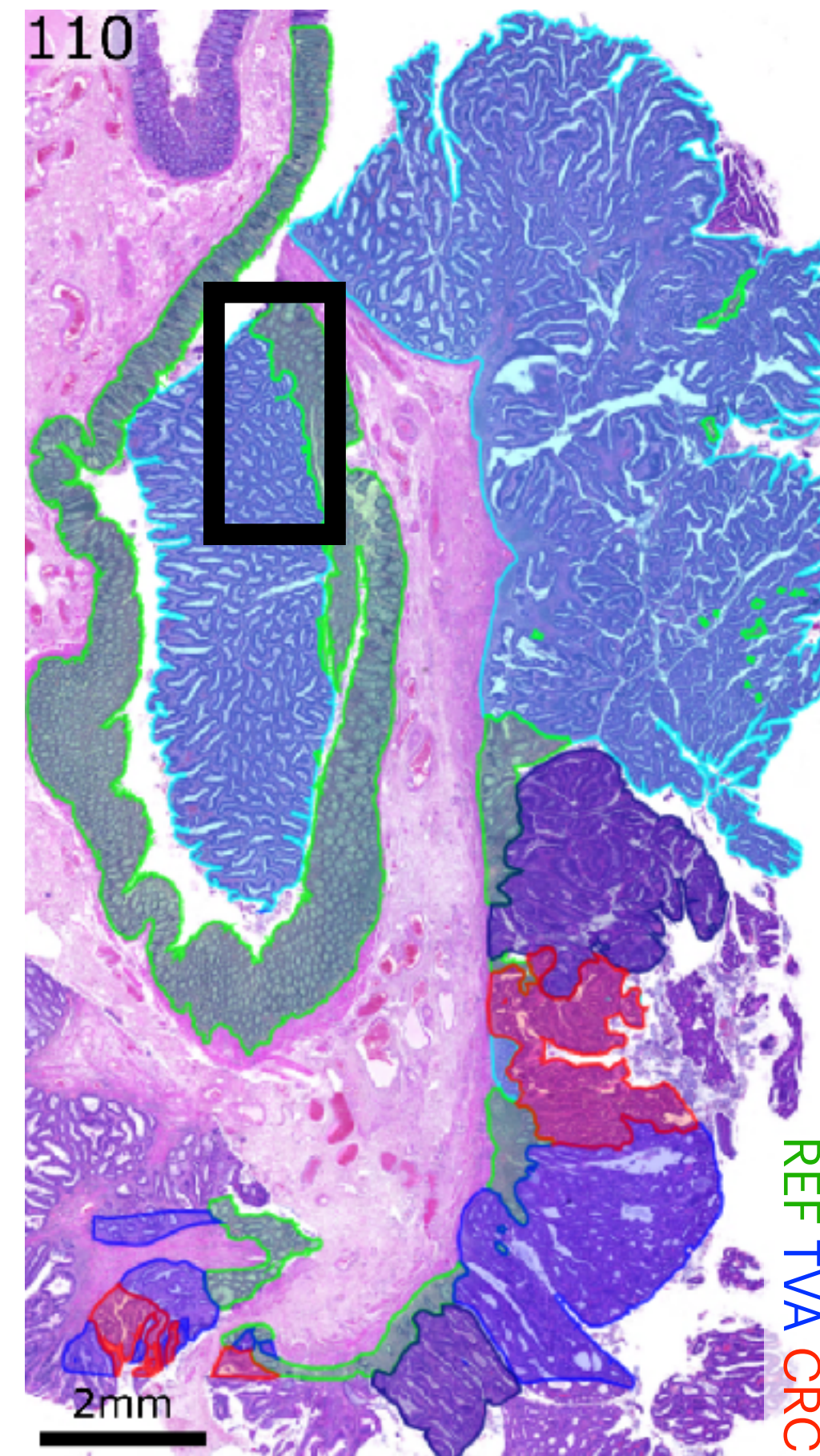
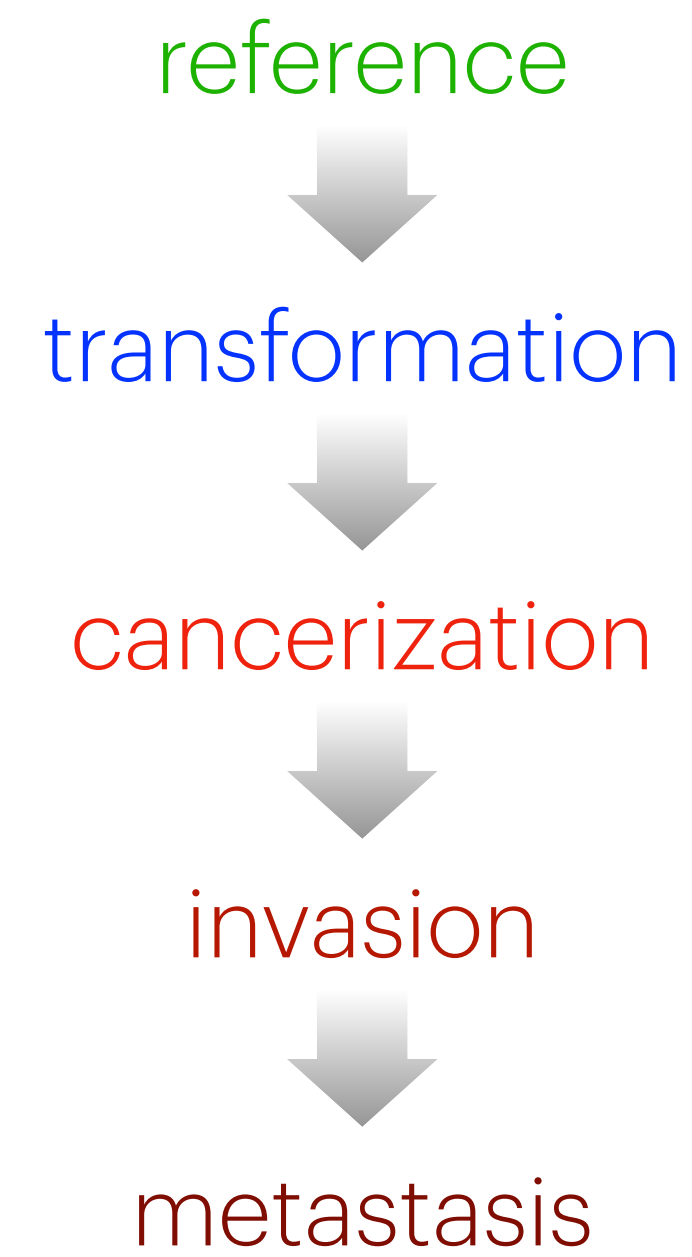
6k-plex CosMx on
TMA of lymphoma

single-cell
FACS
scRNA-seq
scATAC-seq
snRNA-seq
snPATHO-seq
10x Multiome
BCR- & TCR-seq
Chromium Connect

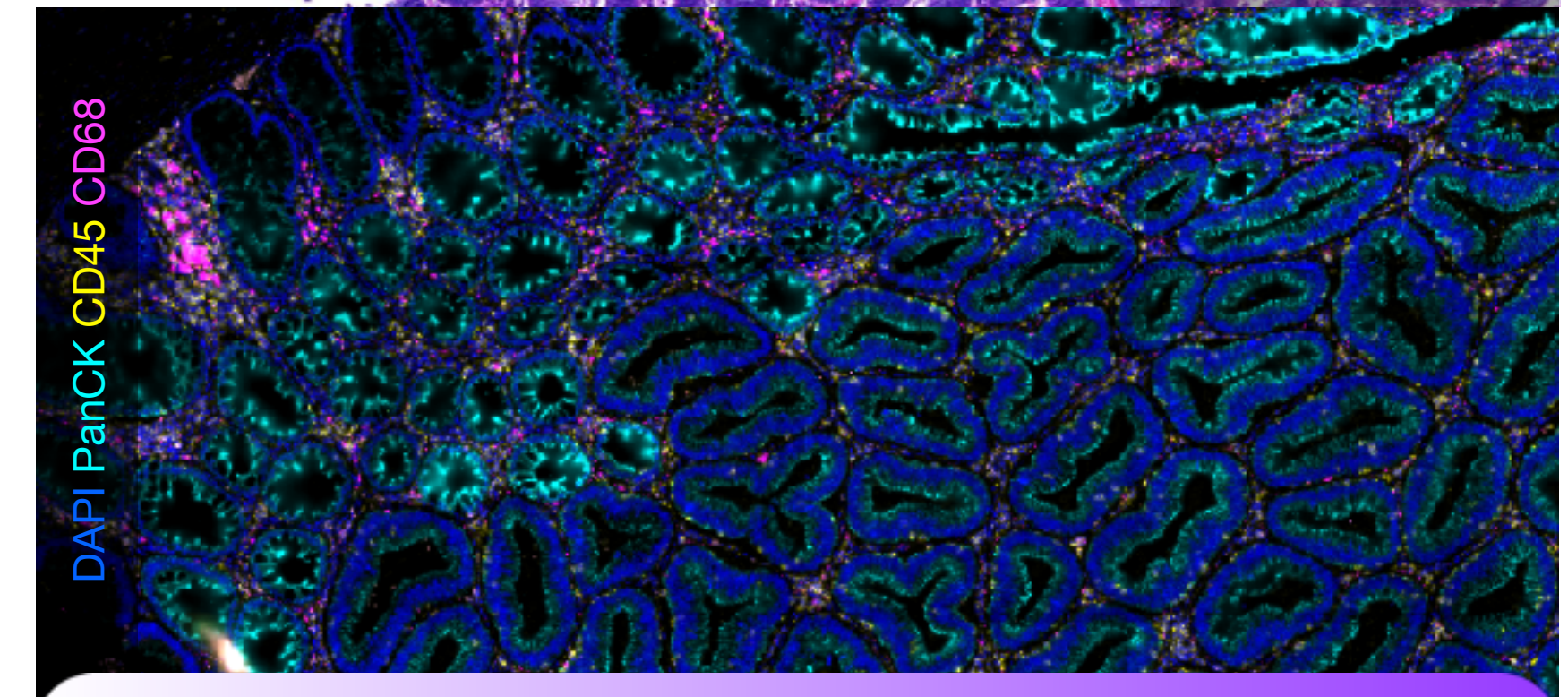
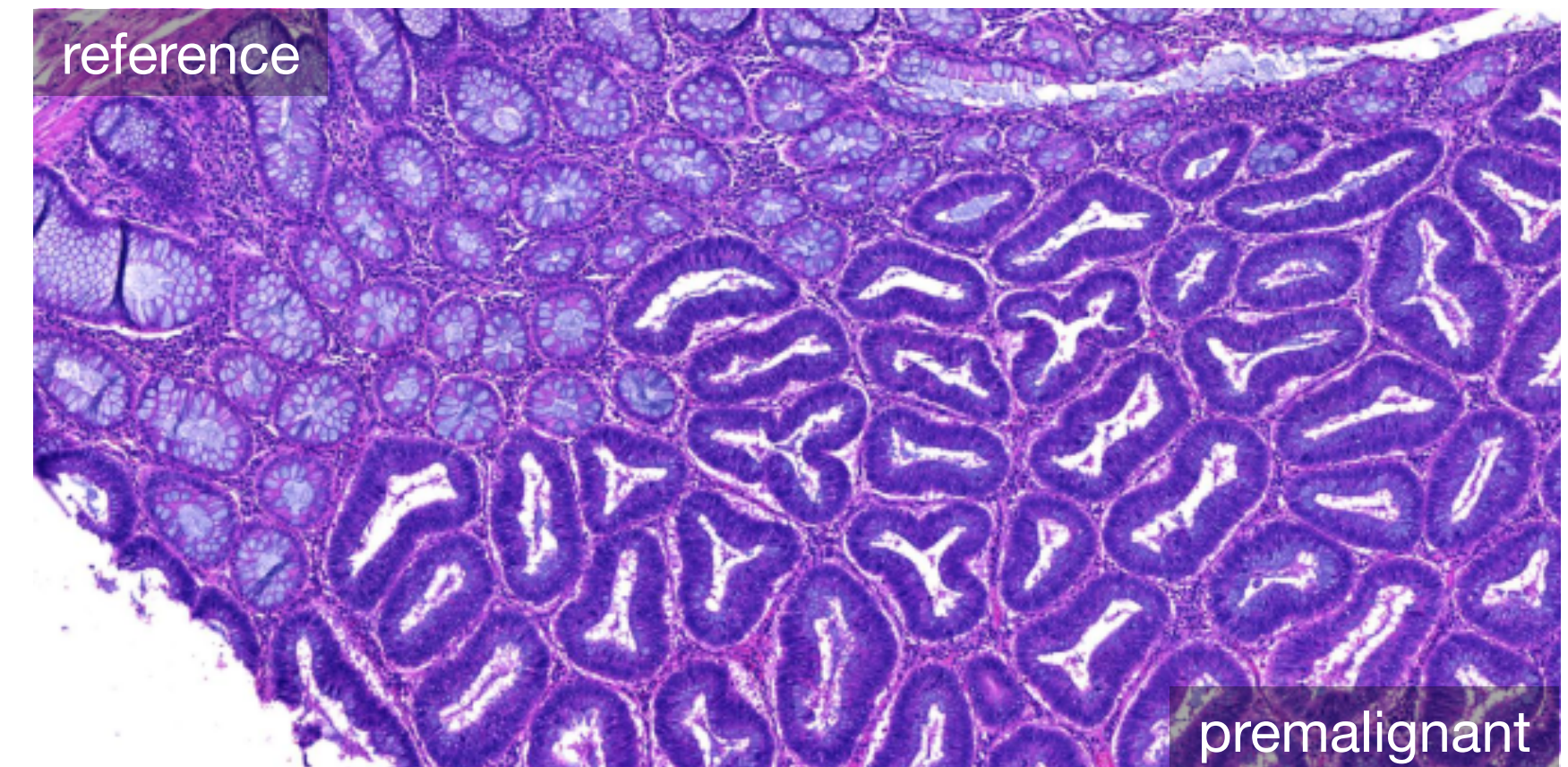
spatial
CosMx & Xenium
Visium & HDST
Stereo-seq



brief intro to colorectal cancer



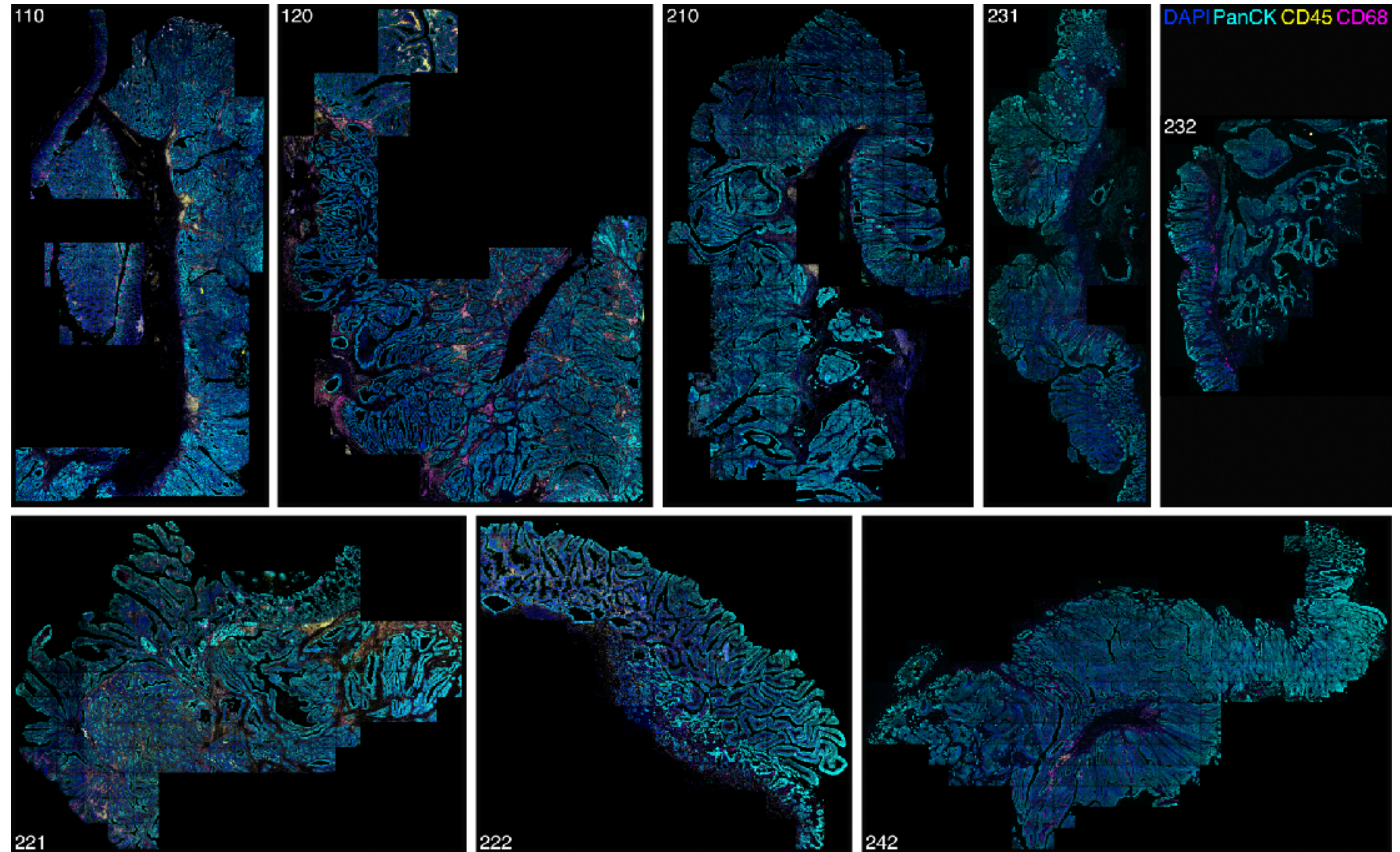
TVA = tubulovillous adenoma
CRC = colorectal cancer



DAPI PanCK CD45 CD68

? REF → TVA → CRC ?
healthy | pre- | malignant

histopathology: REF/TVA/CRC annotation

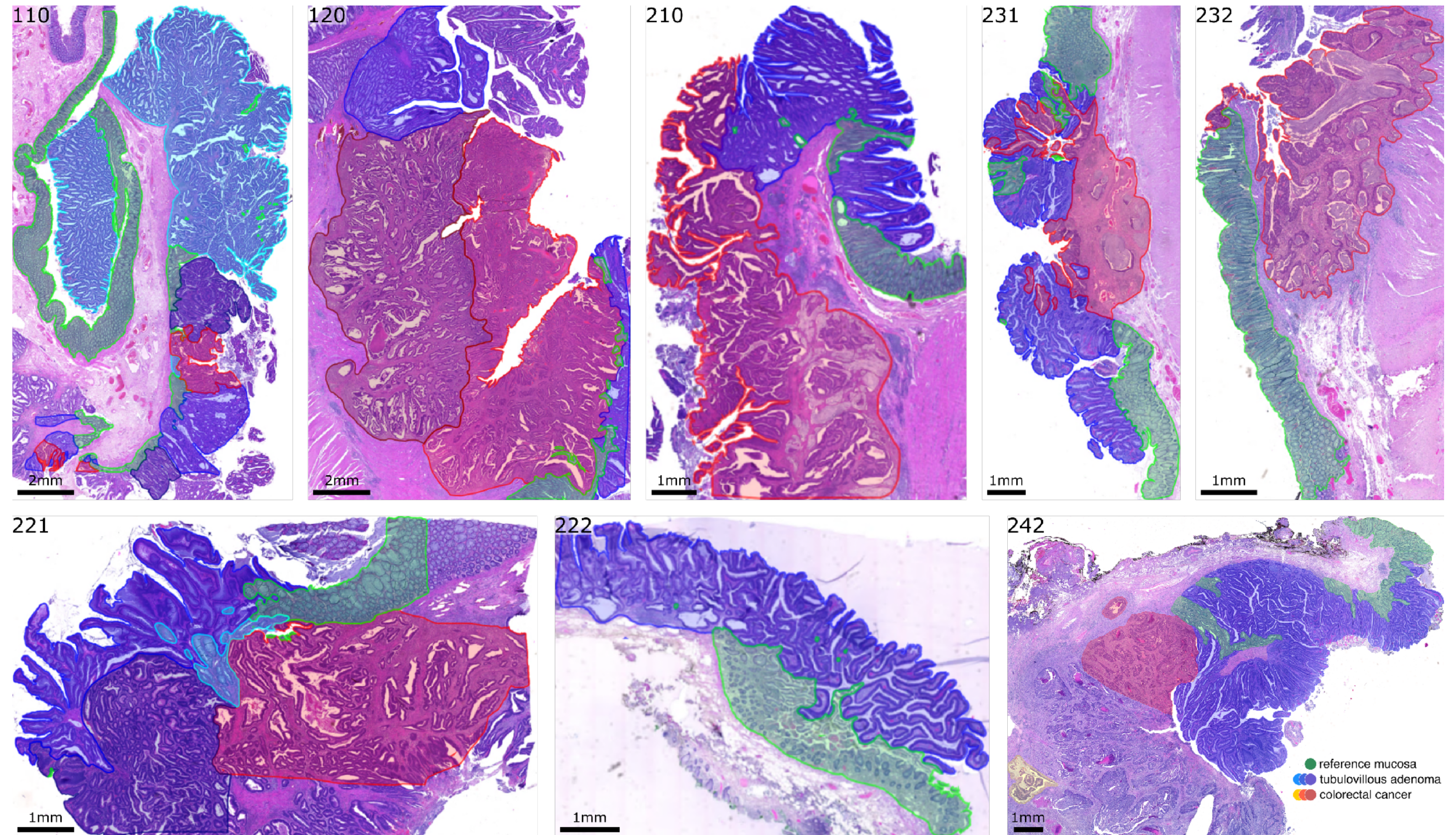


histopathology: REF/TVA/CRC annotation

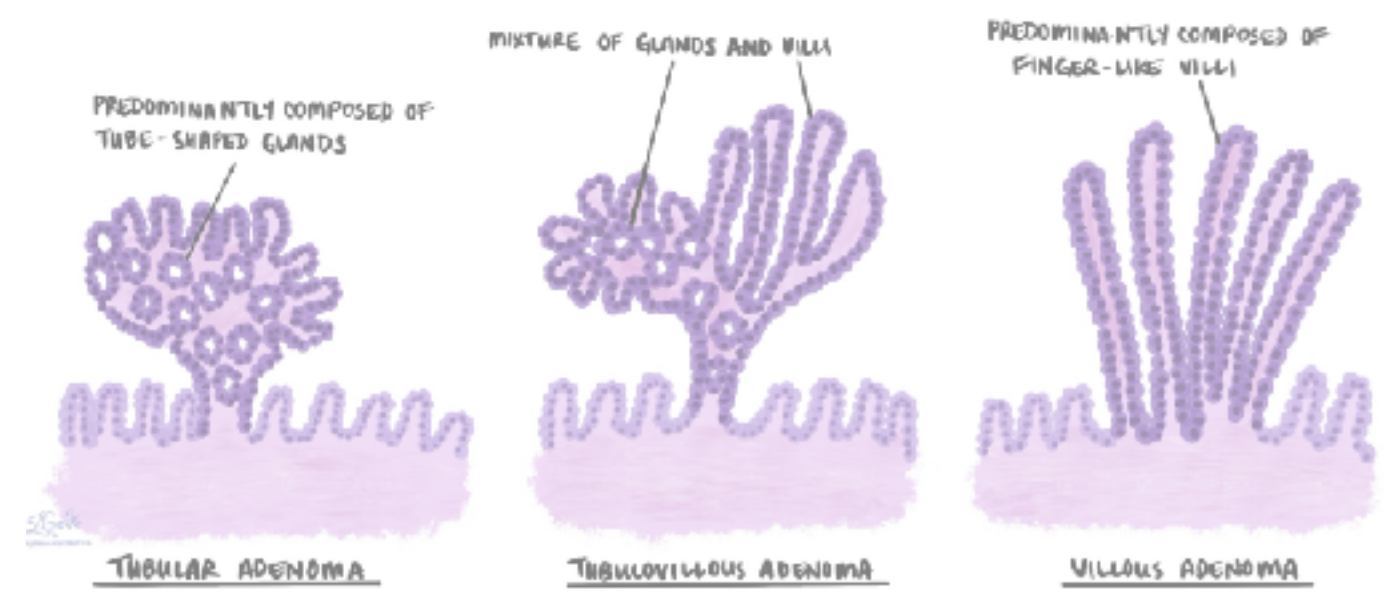
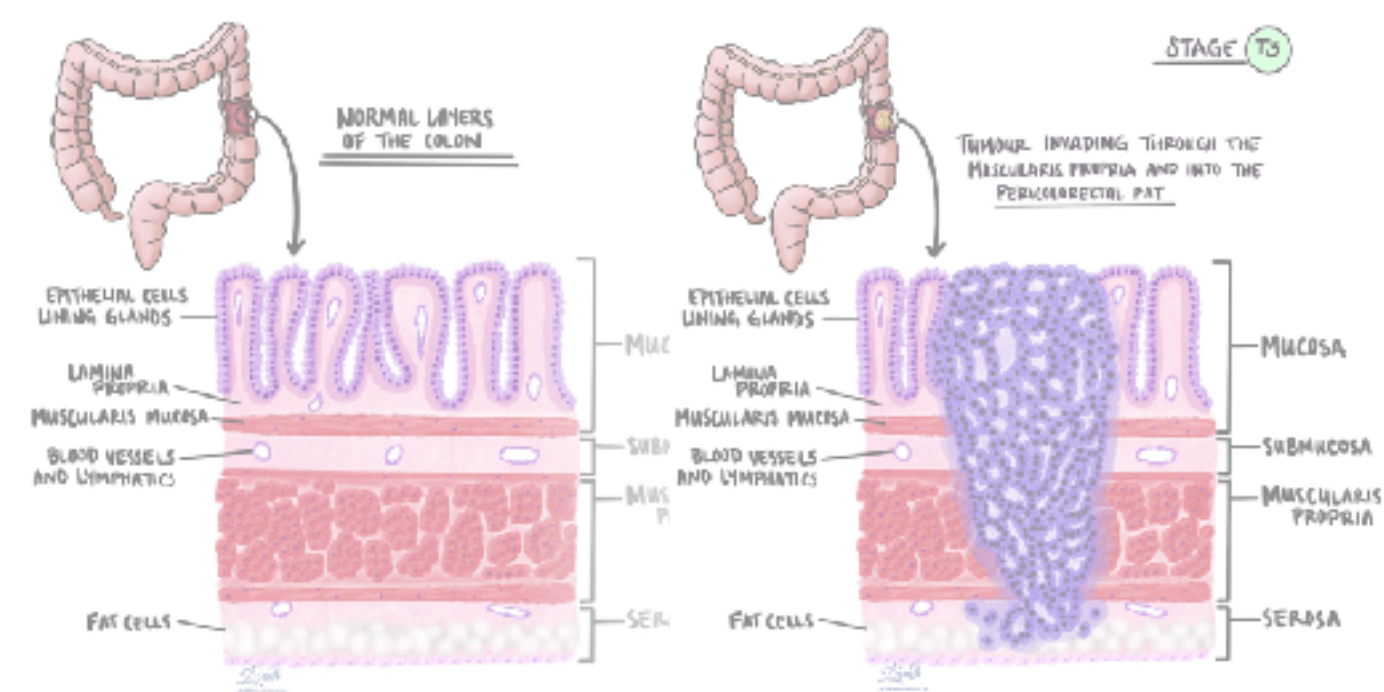
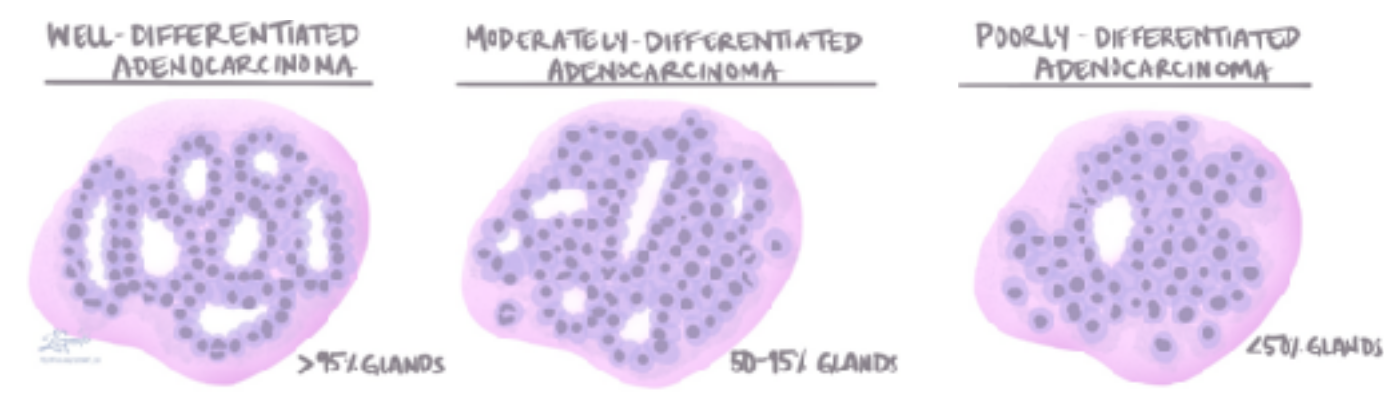
healthy reference mucosa (REF)
pre- tubulovillous adenoma (TVA)
malignant colorectal cancer (CRC)



27 regions across sections
(1 REF, 0-3 TVA, 0-2 CRC each)



histopathology: REF/TVA/CRC annotation

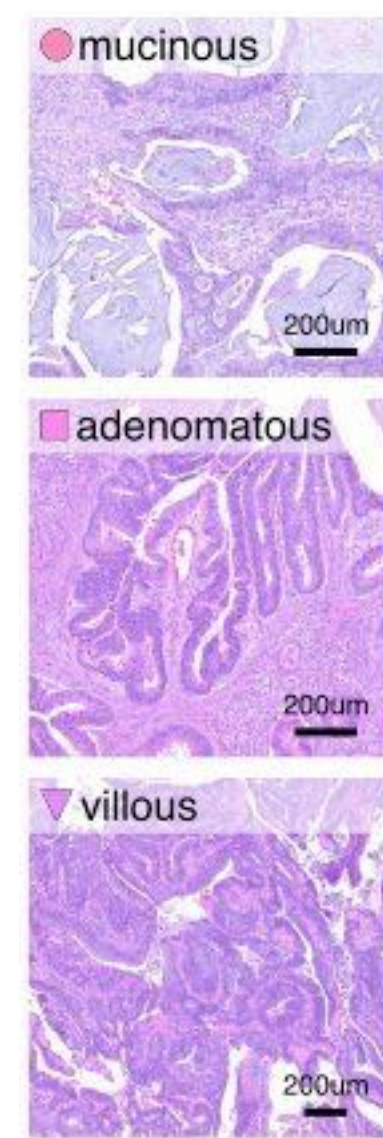


grading

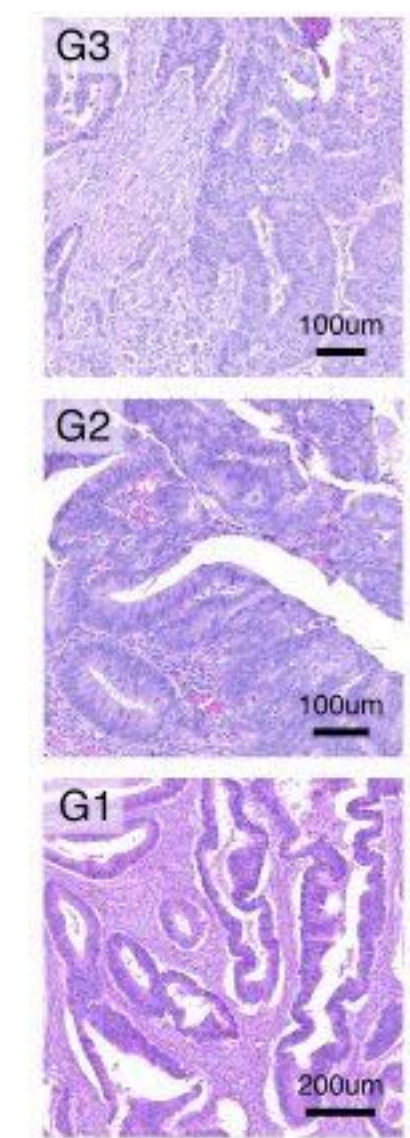
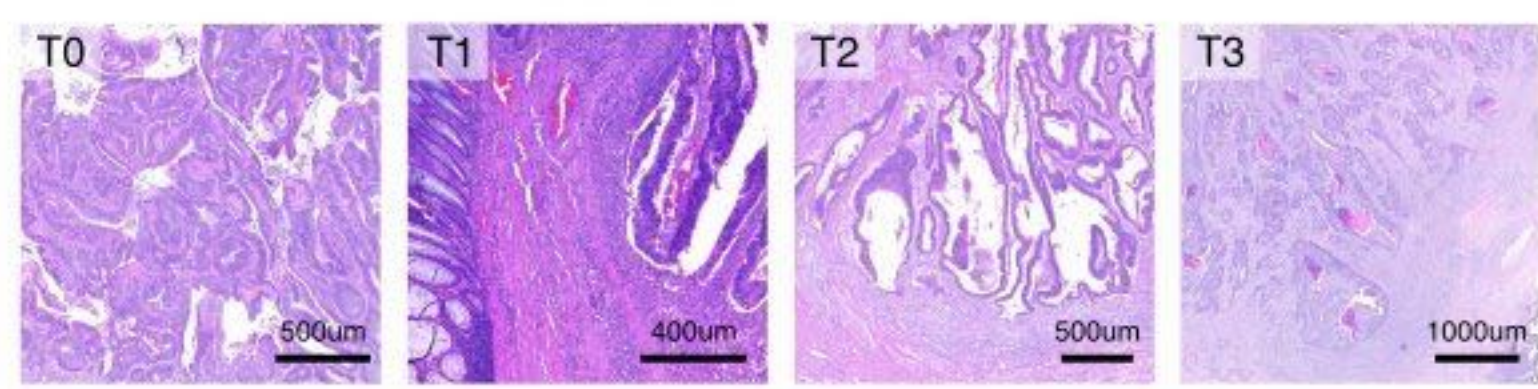
staging

features

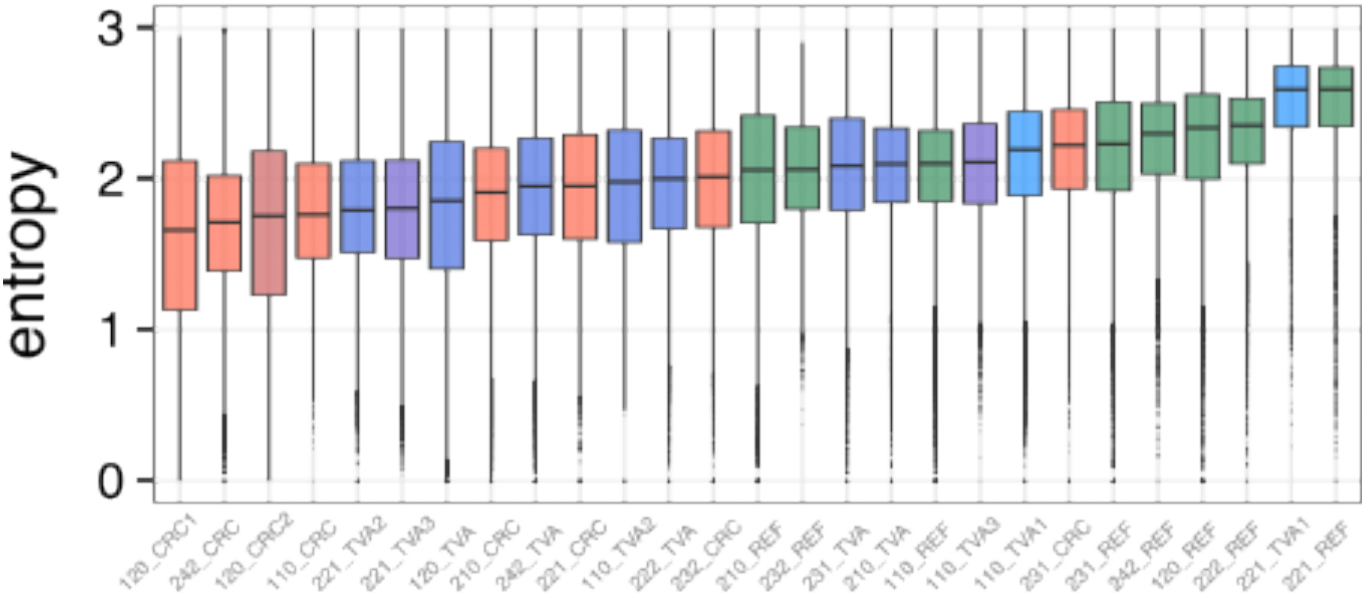
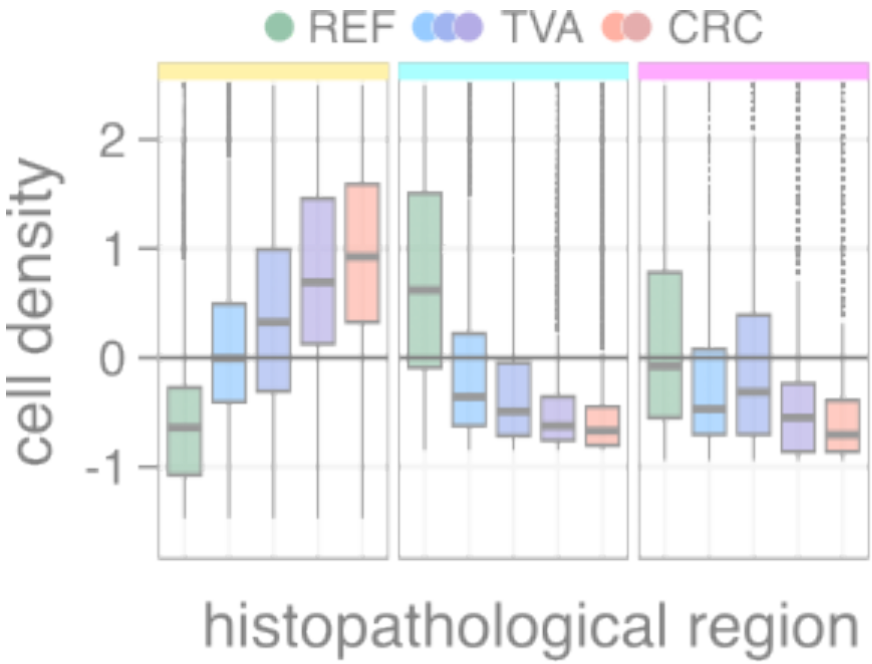
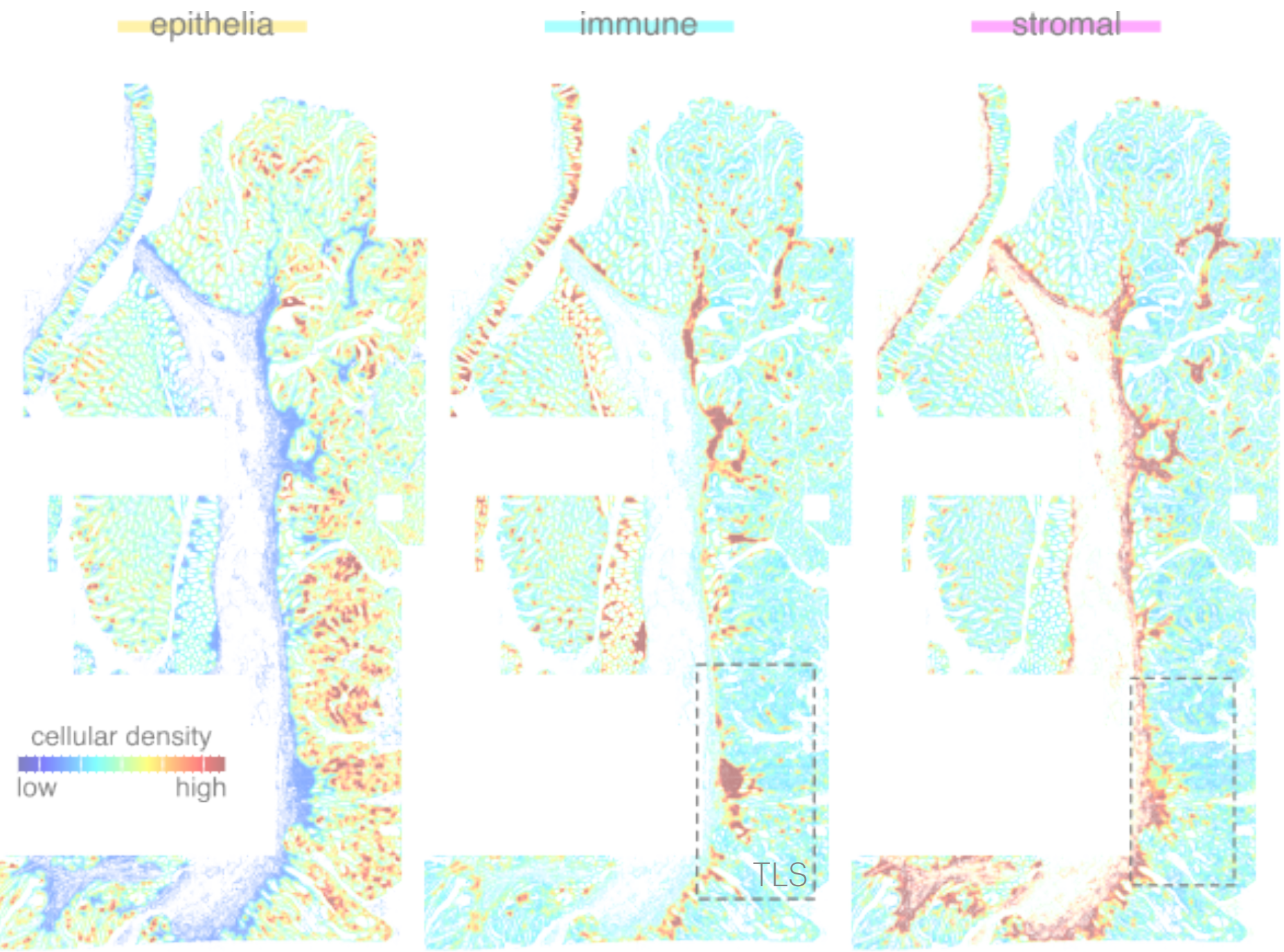
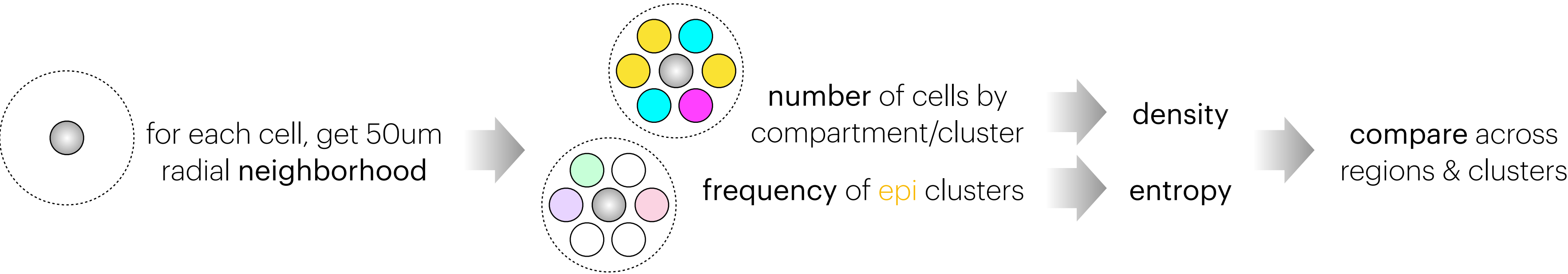
- inflammation
- angiogenesis
- growth pattern
- lymphovascular invasion
- tertiary lymphoid structure
- ...



				primary	metastasis
				grading-differentiation	
staging-invasion	T0	T1	T2	T3	
					G3
					G2
	T0	T1	T2	T3	G1
	110	321	521	242	412
	321	120	210		411
	323	322	120		512
			221		



neighborhoods: epi. become dense & homogeneous



low/high H = more homo/heterogeneous
(e.g., one cluster -> H = 0; all equal -> H = 1)

trajectory of malignant transformation through space

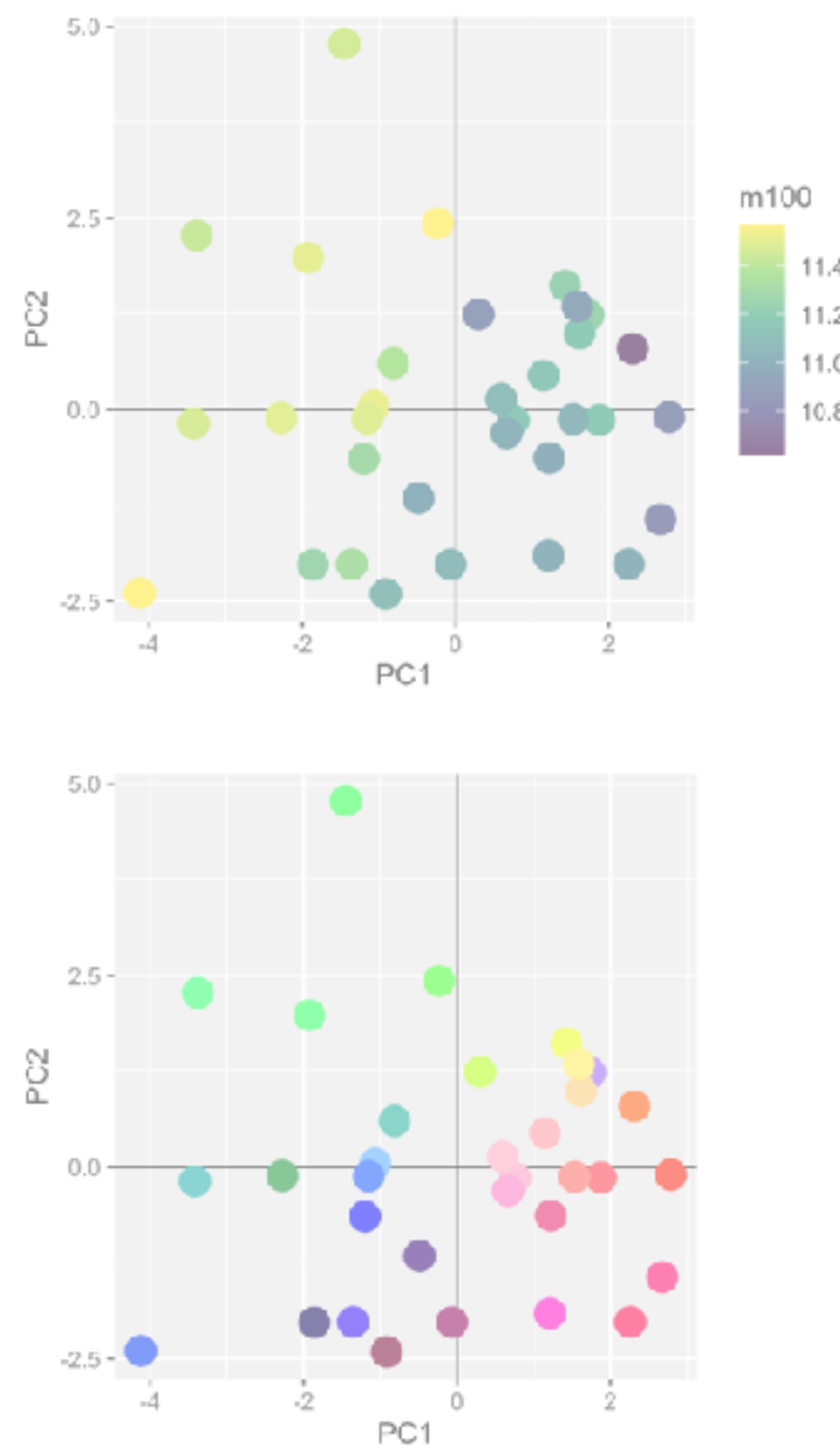
'decathlon' dataset



points = athletes,
color = 100m time (s)



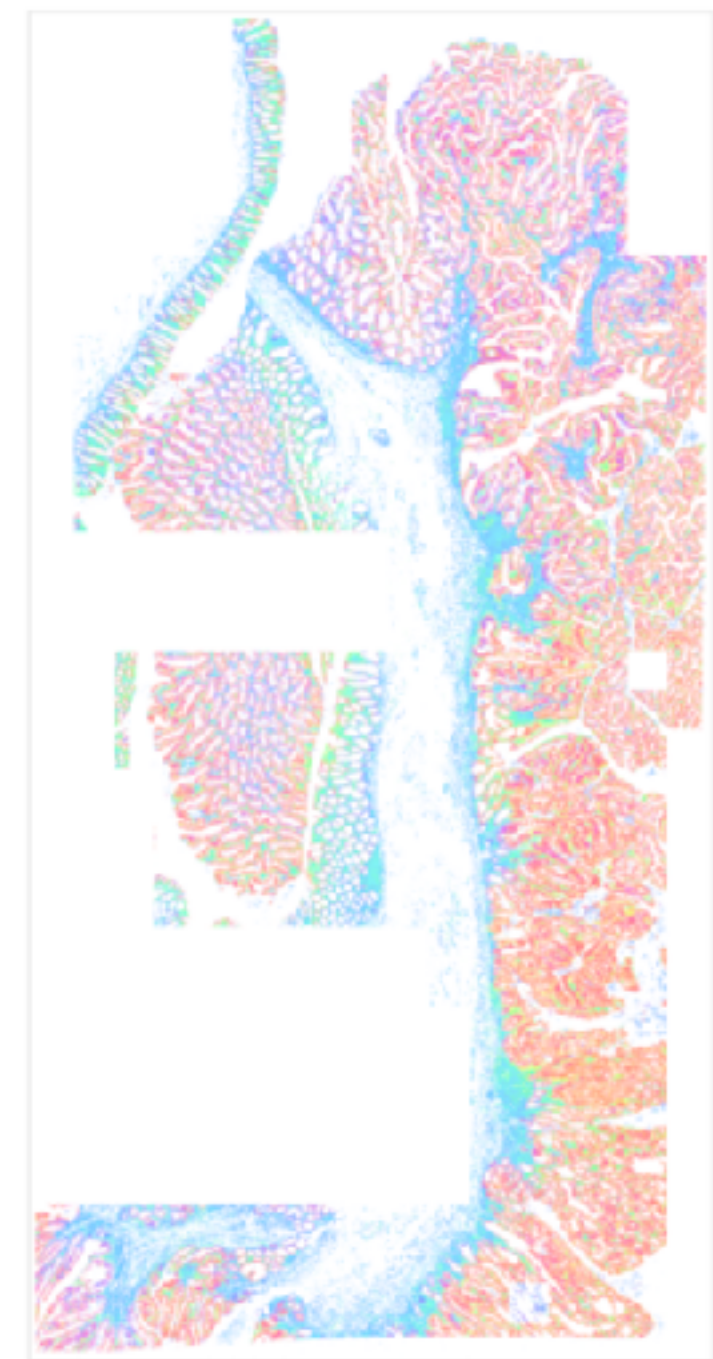
map PC1-3 to 0-255
& color as RGB



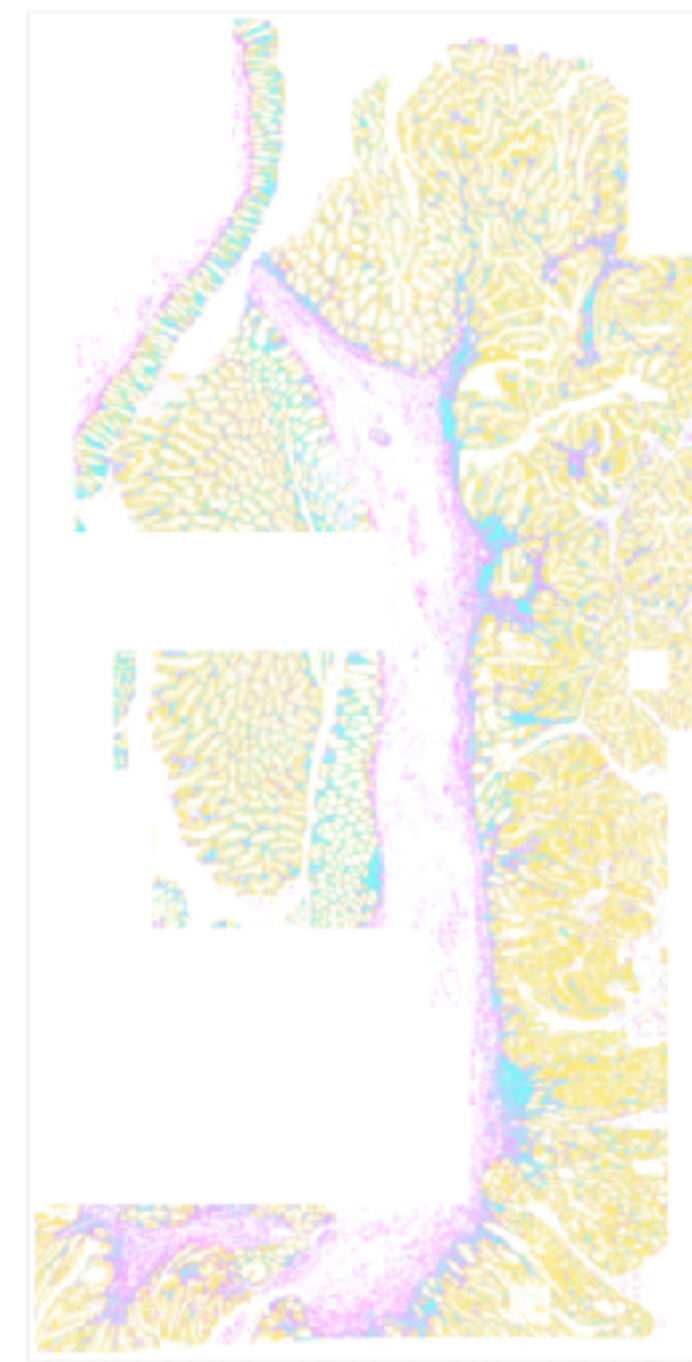
PC1-3 capture compartments



PC1-3 of **epi** capture malignant transformation



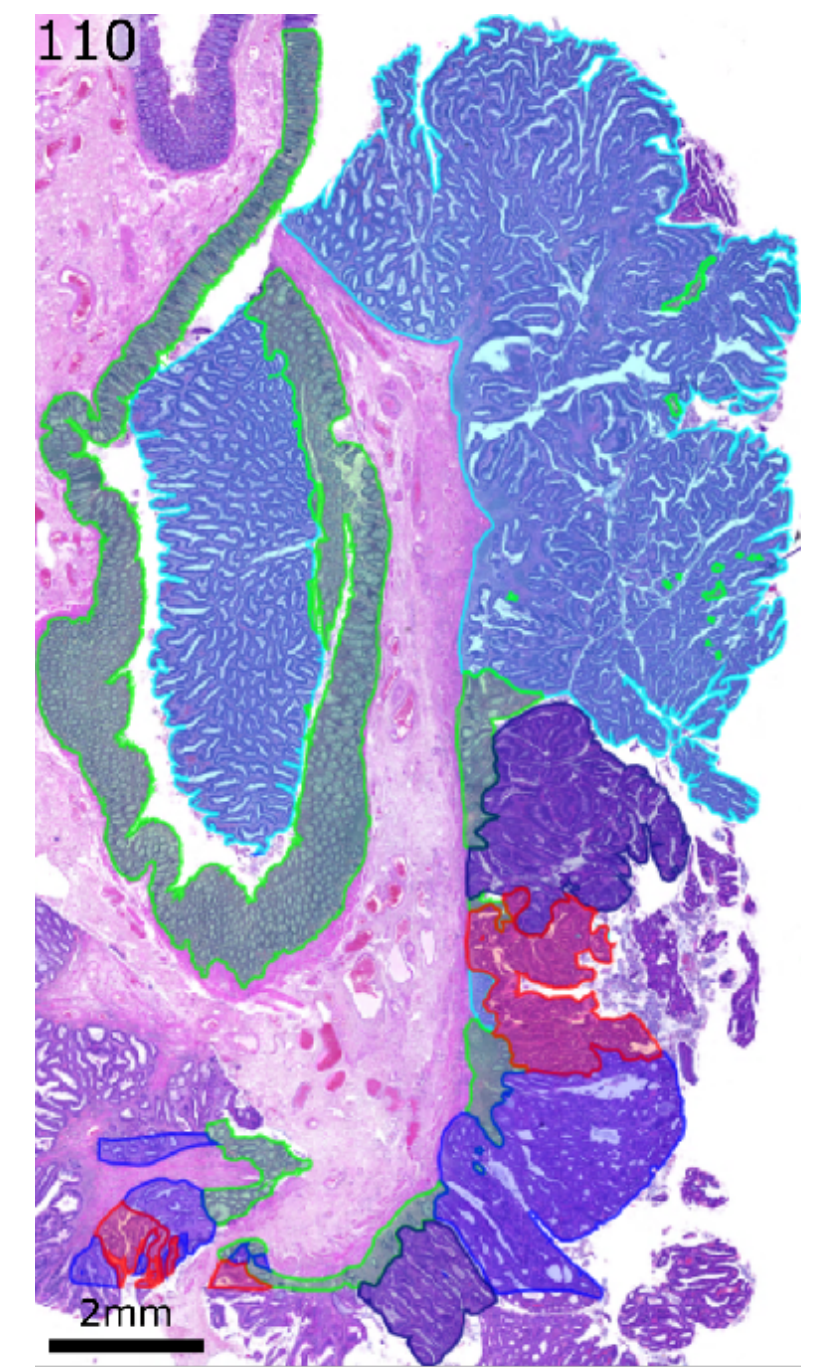
epi imm str



epi imm str

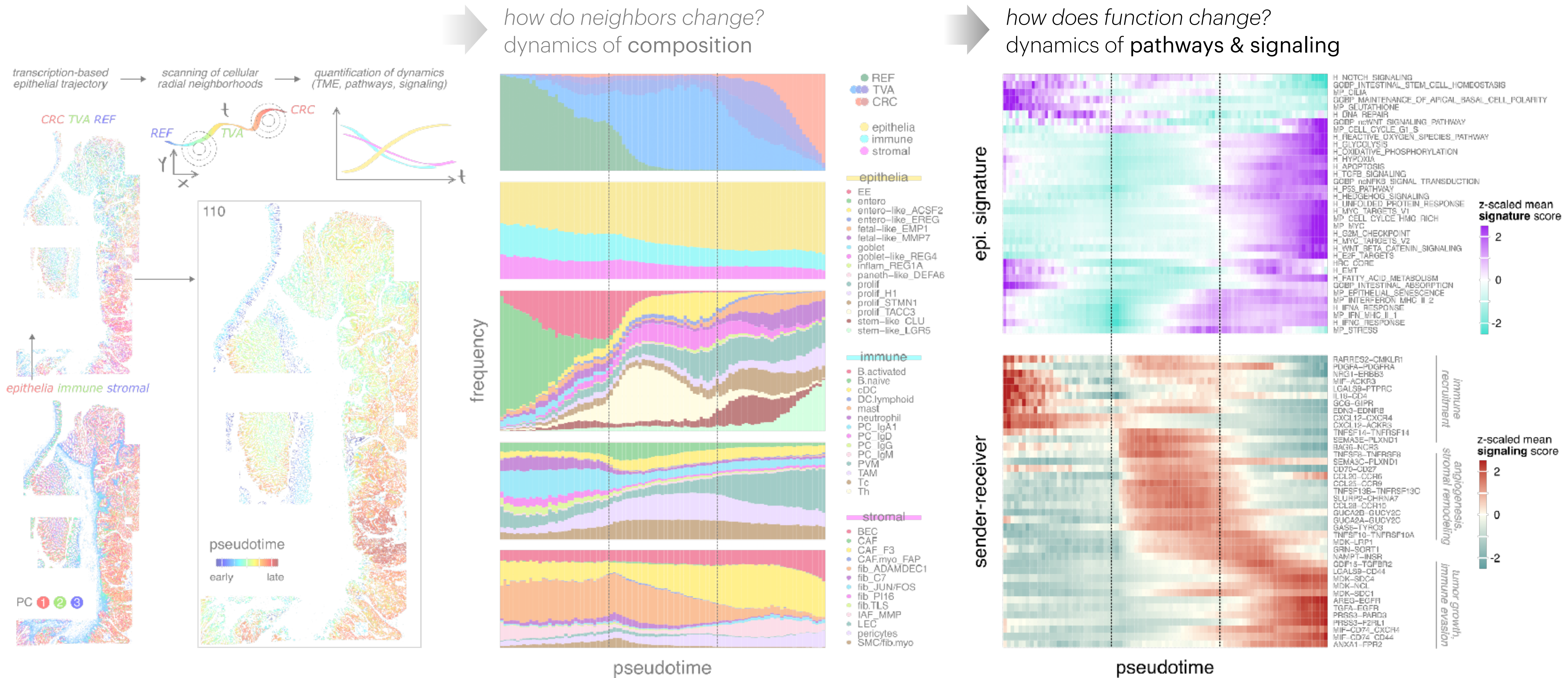


crc tva ref

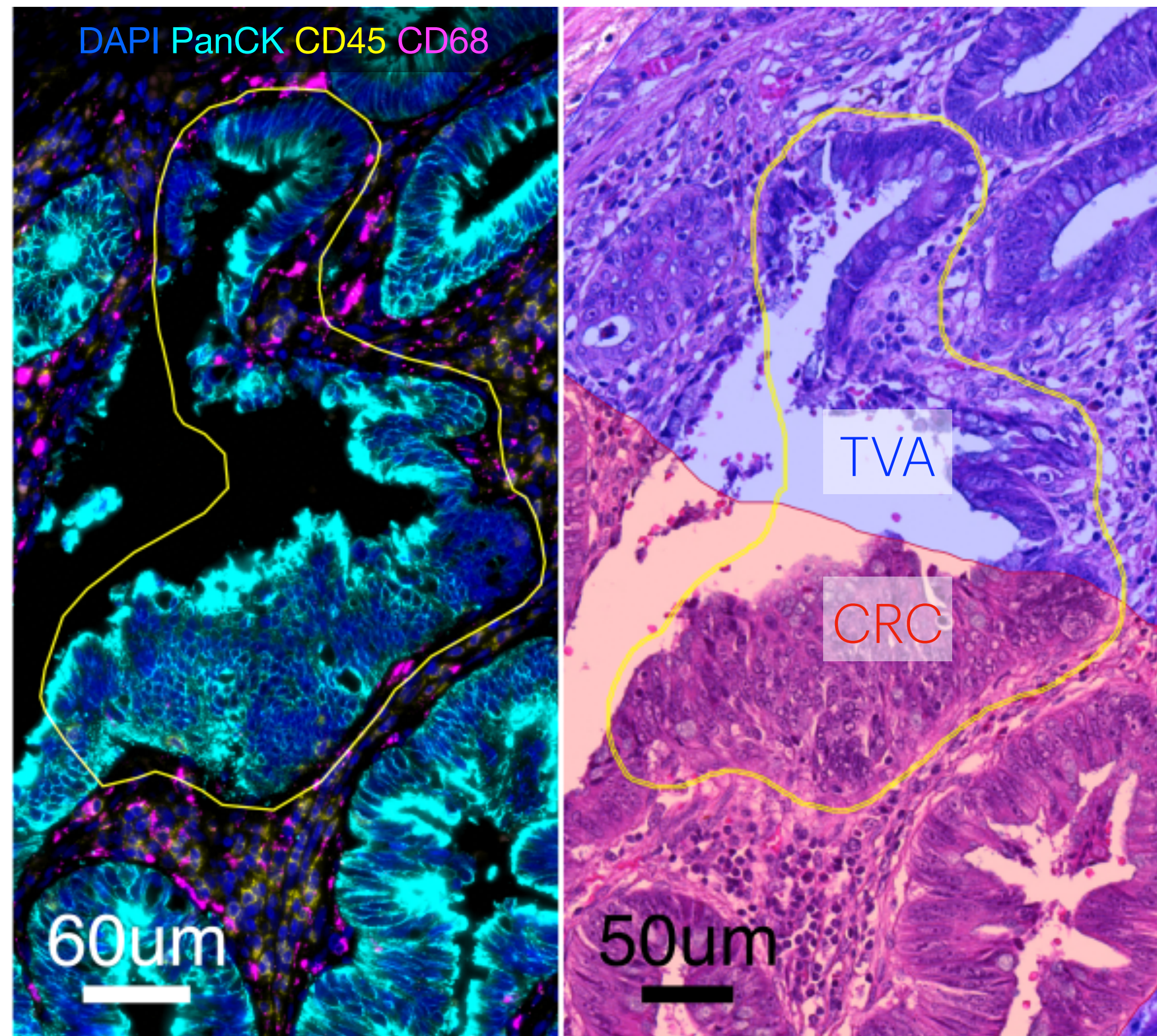


● reference mucosa
● tubulovillous adenoma
● colorectal cancer

dynamics of malignant transformation through space



transition crypts: zooming in on transformation



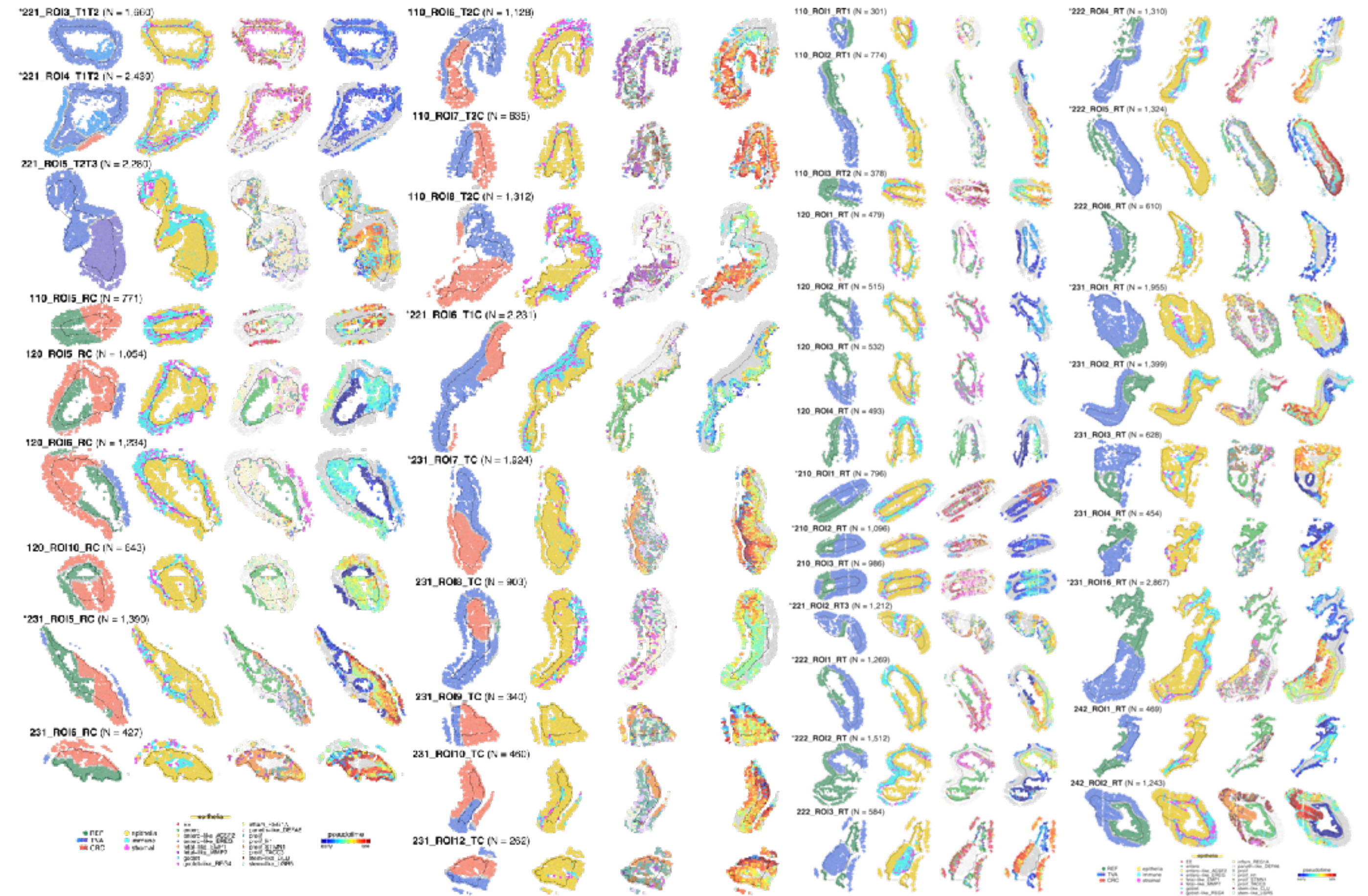
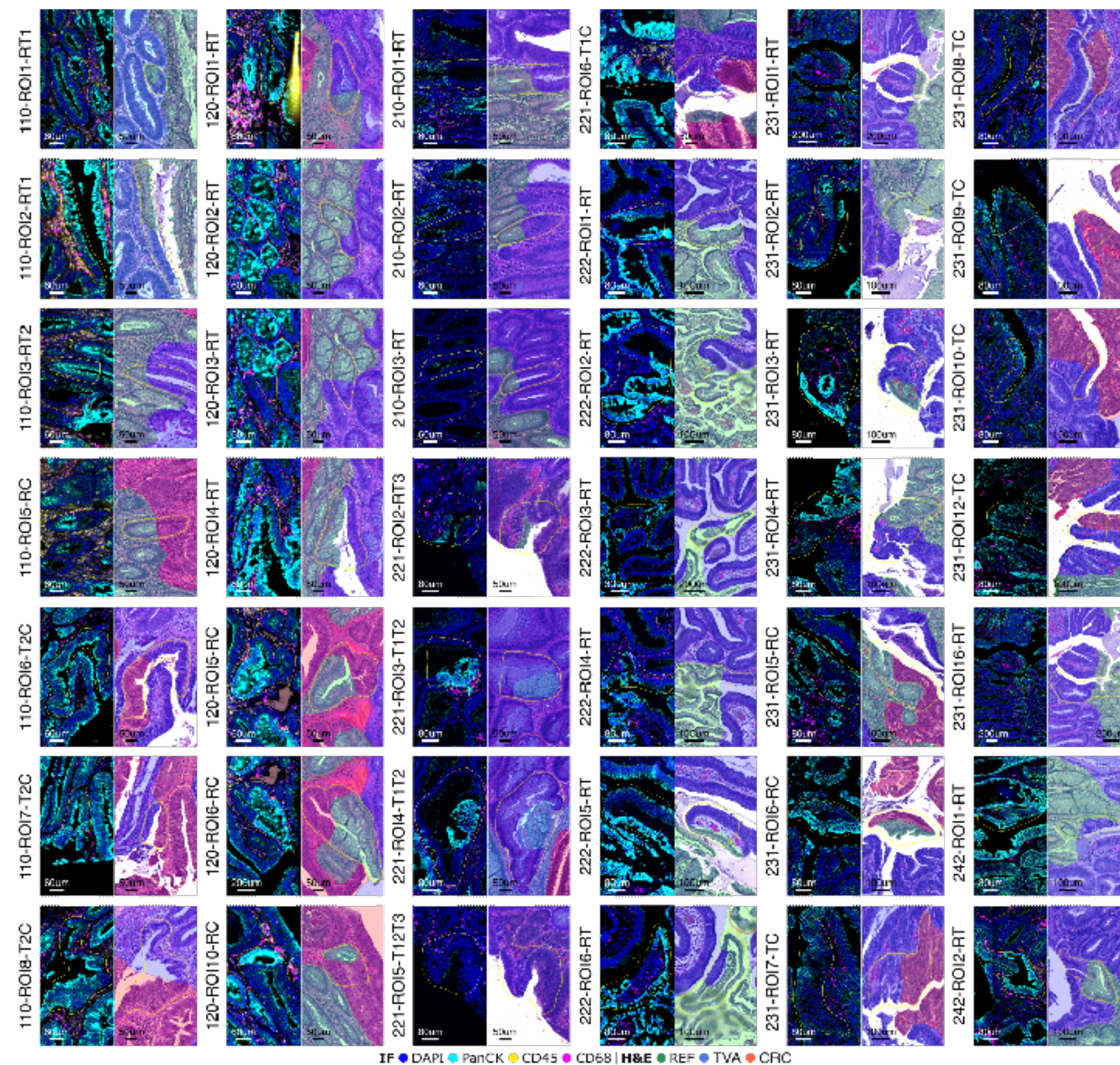
- ◆ single colonic crypt w/
divided histopathological
& transcriptional makeup
- ◆ from rare crypt **fusion**, or an
abrupt **transformation** event

transition crypts: zooming in on transformation

42 transition crypts
(24 RT, 6 RC, 9 TC, 3 TT)

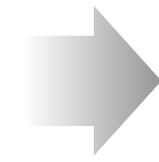


selection + 50um expansion colored by (fltr)
region, compartment, subpopulation, pseudotime

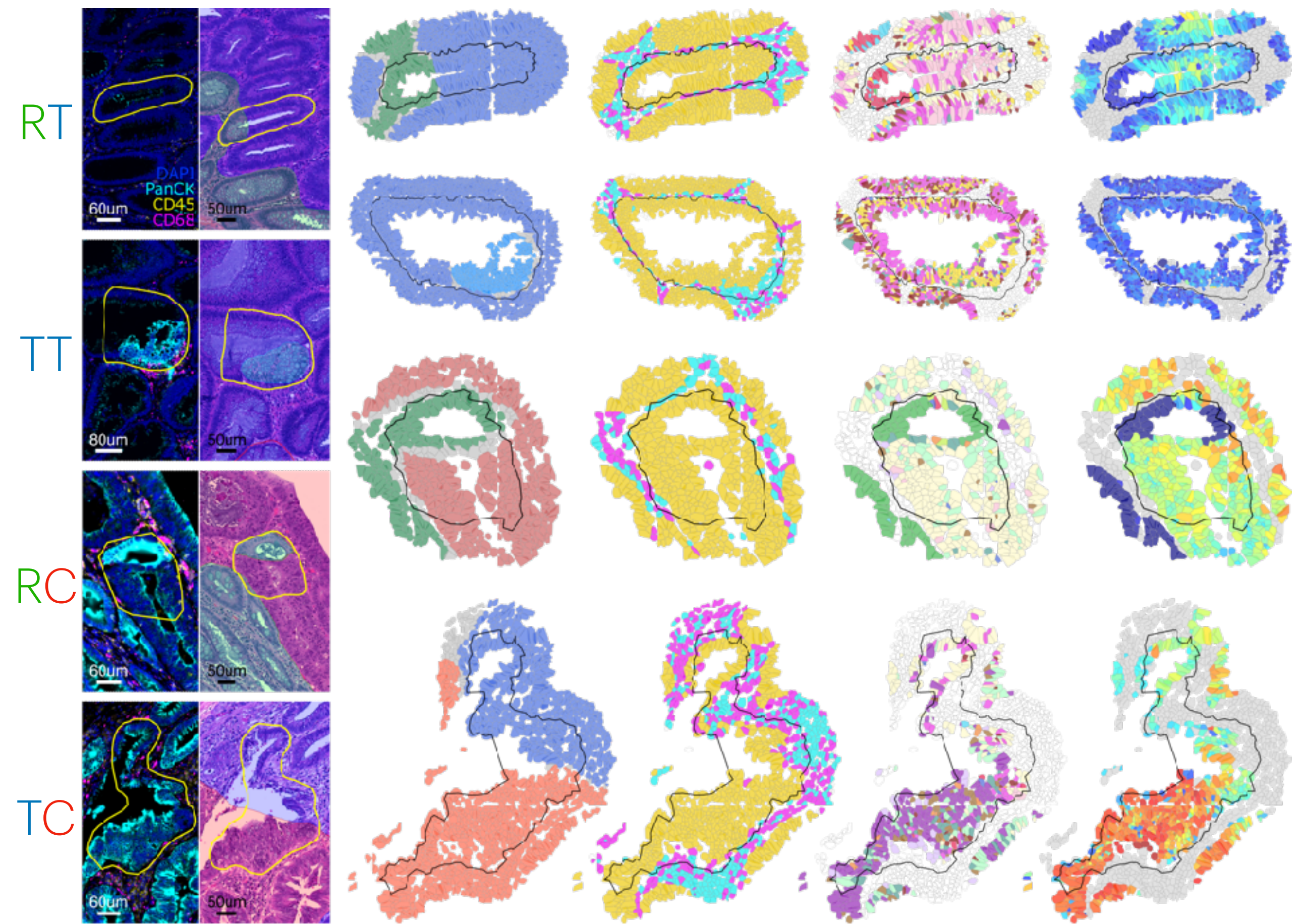
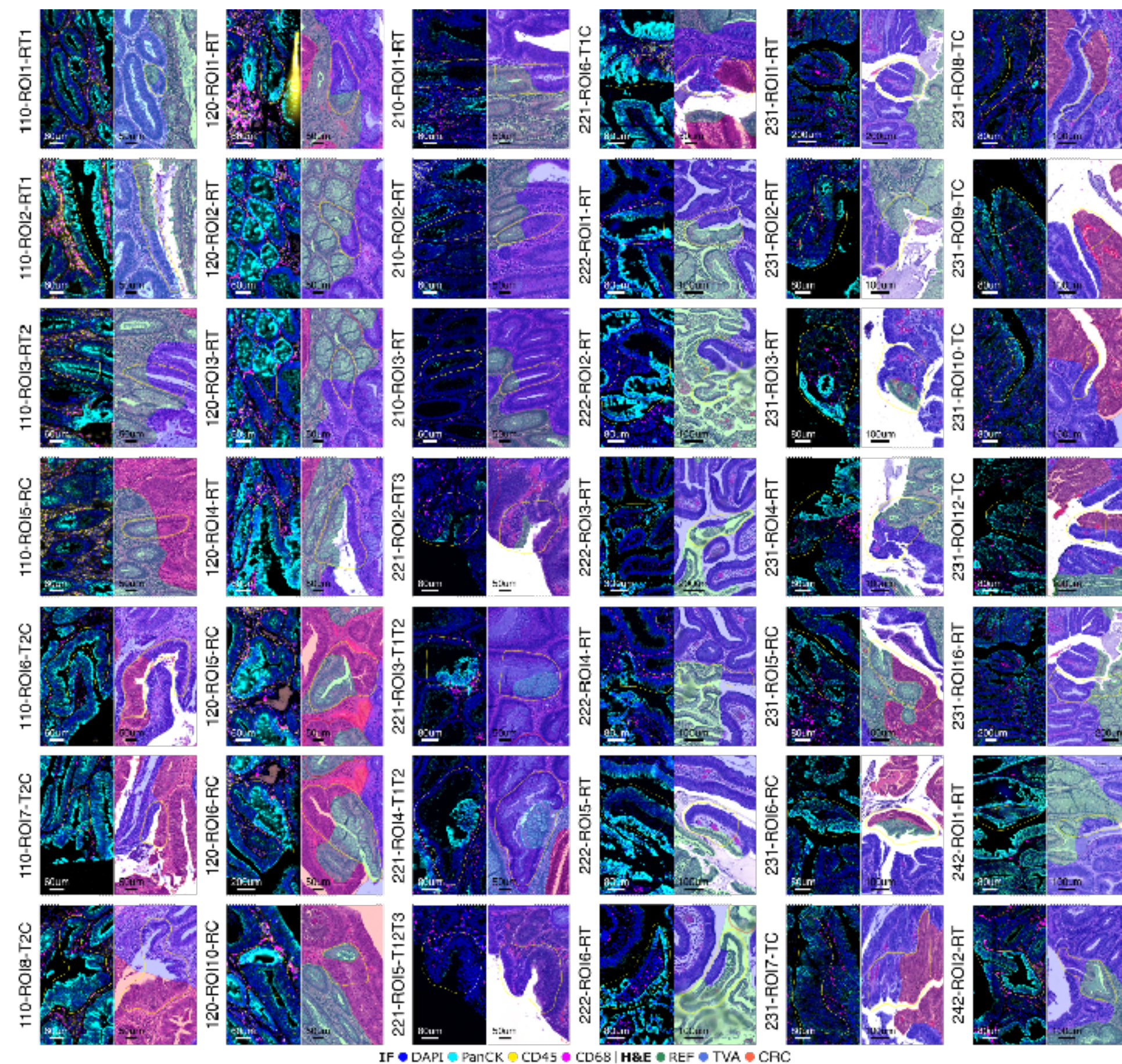


transition crypts: zooming in on transformation

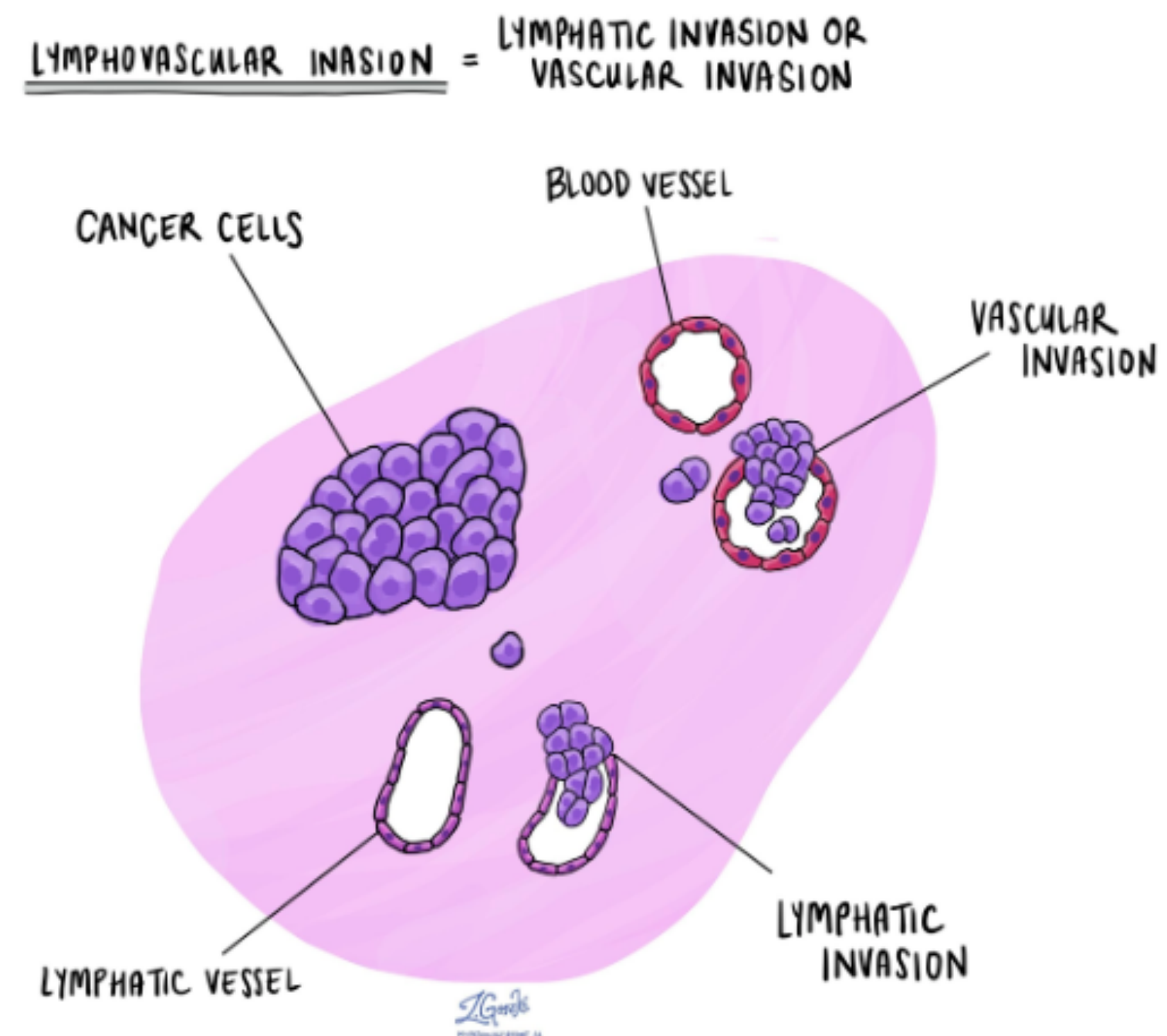
42 transition crypts
(24 RT, 6 RC, 9 TC, 3 TT)



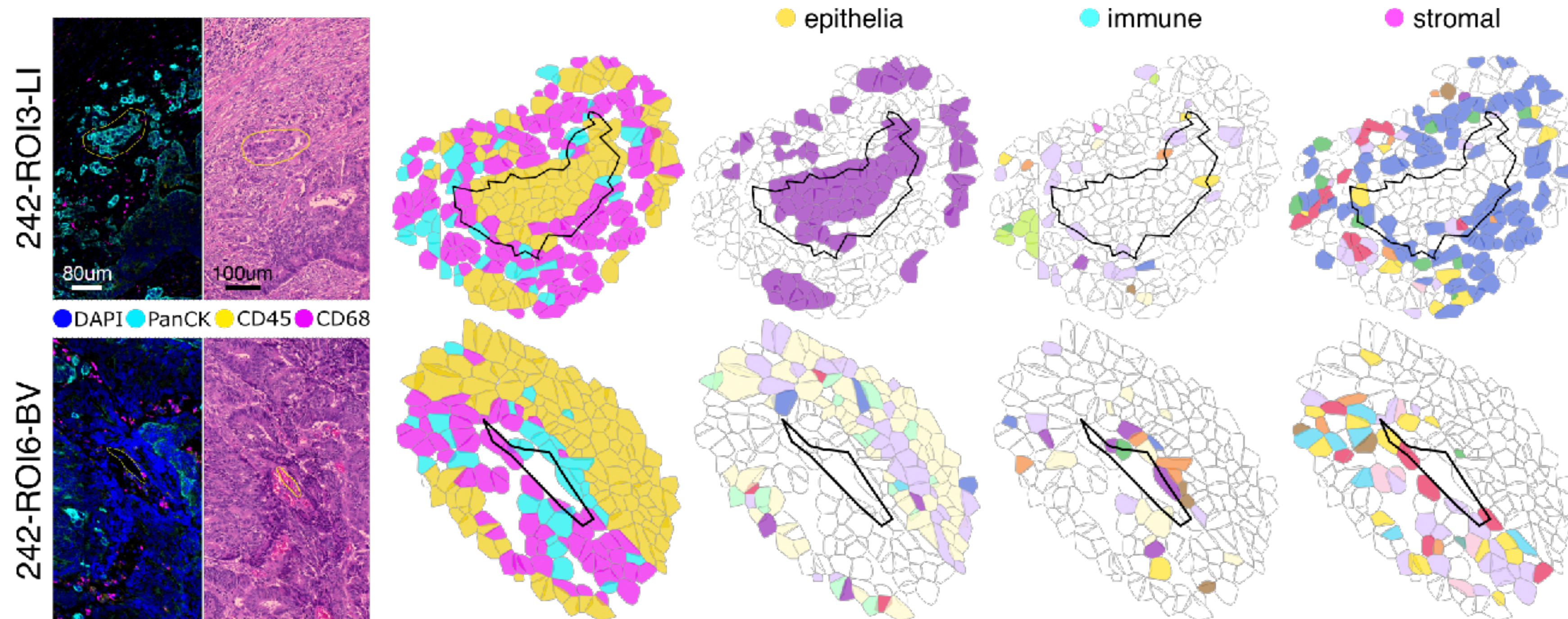
selection + 50um expansion colored by (fltr)
region, compartment, subpopulation, pseudotime



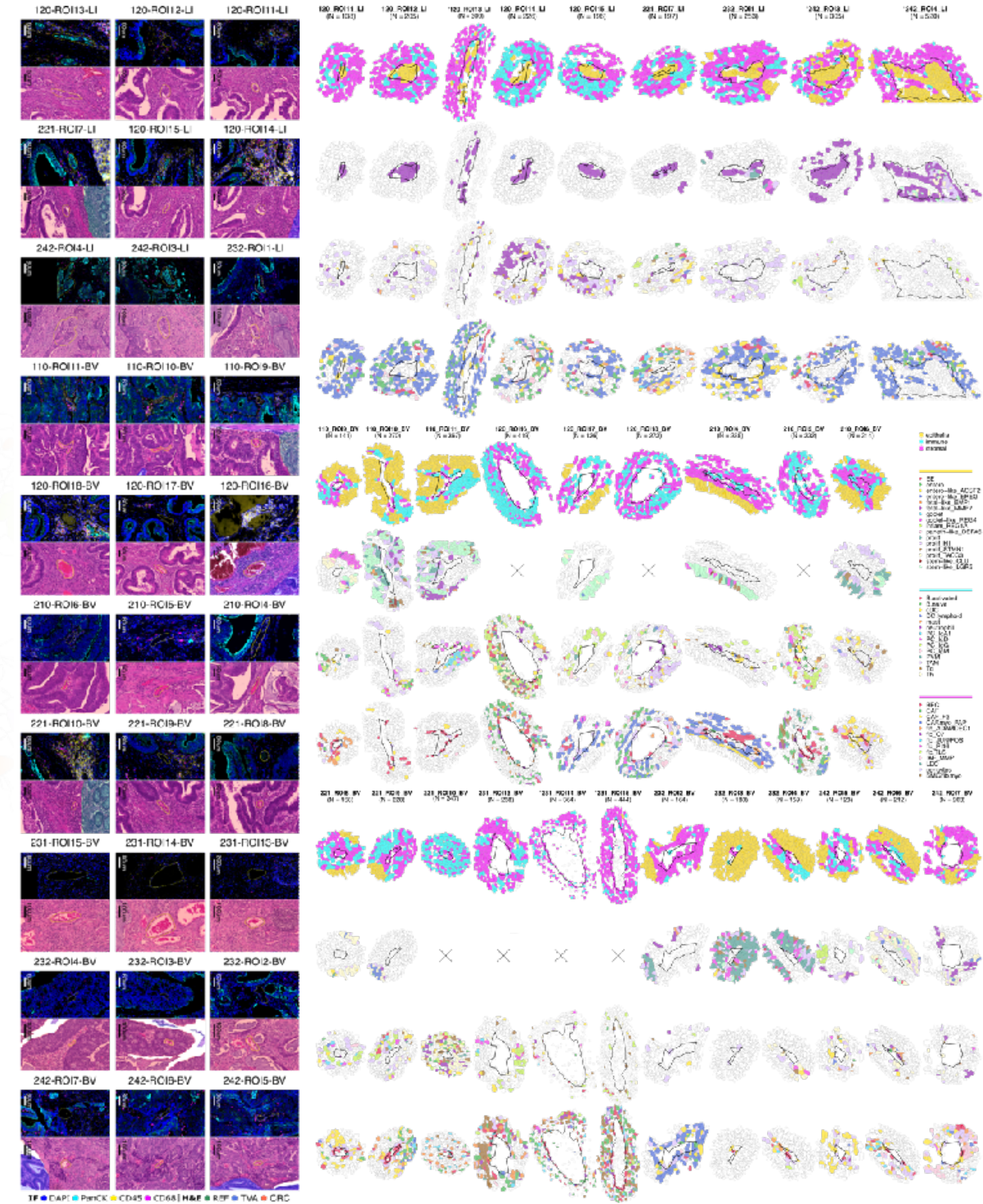
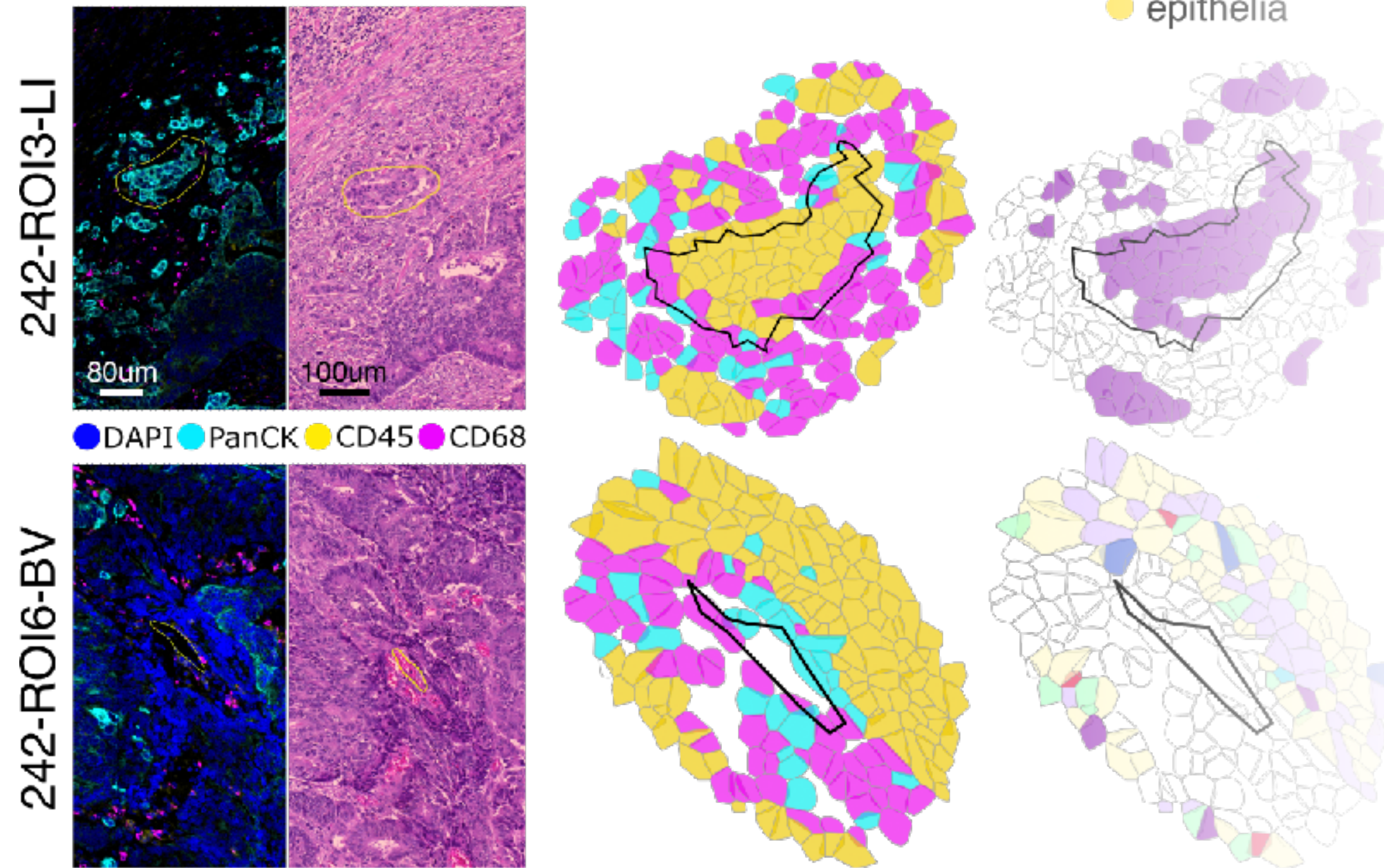
lymphovascular invasions: from growth to metastatic spread



lymphovascular invasions: from growth to metastatic spread

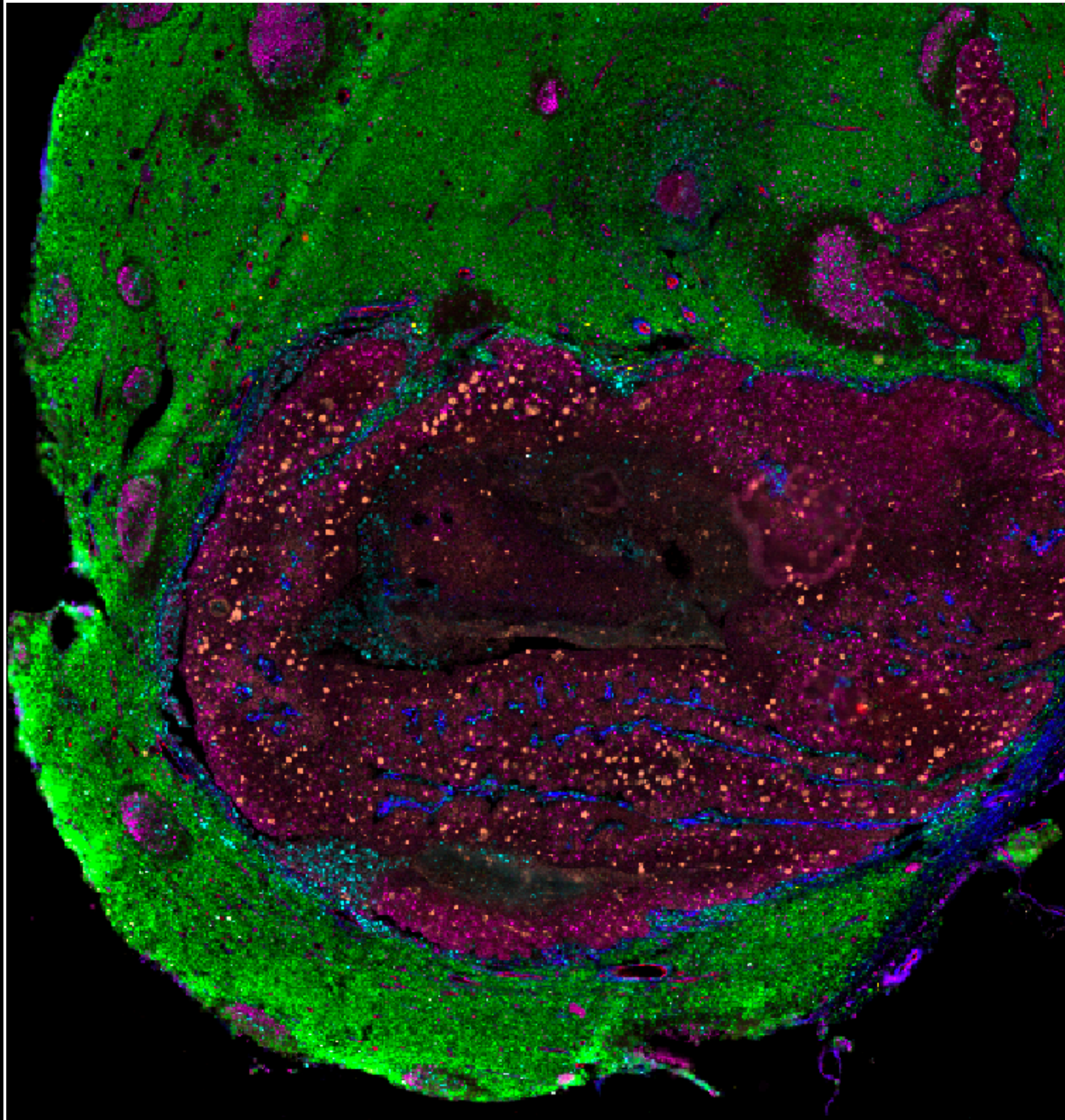


lymphovascular invasions: from growth to metastatic spread

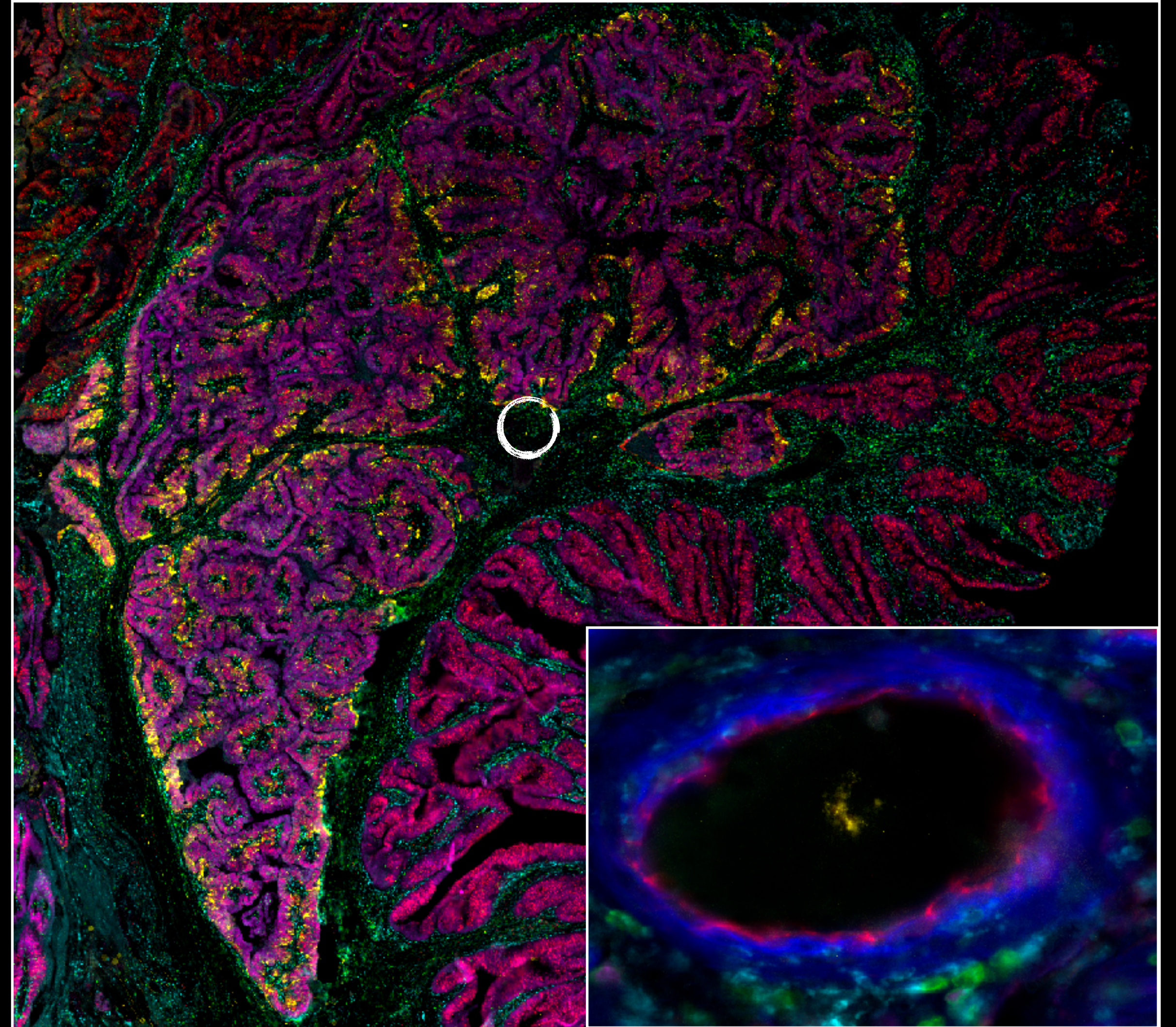


transcripts != proteins

lymph node metastasis

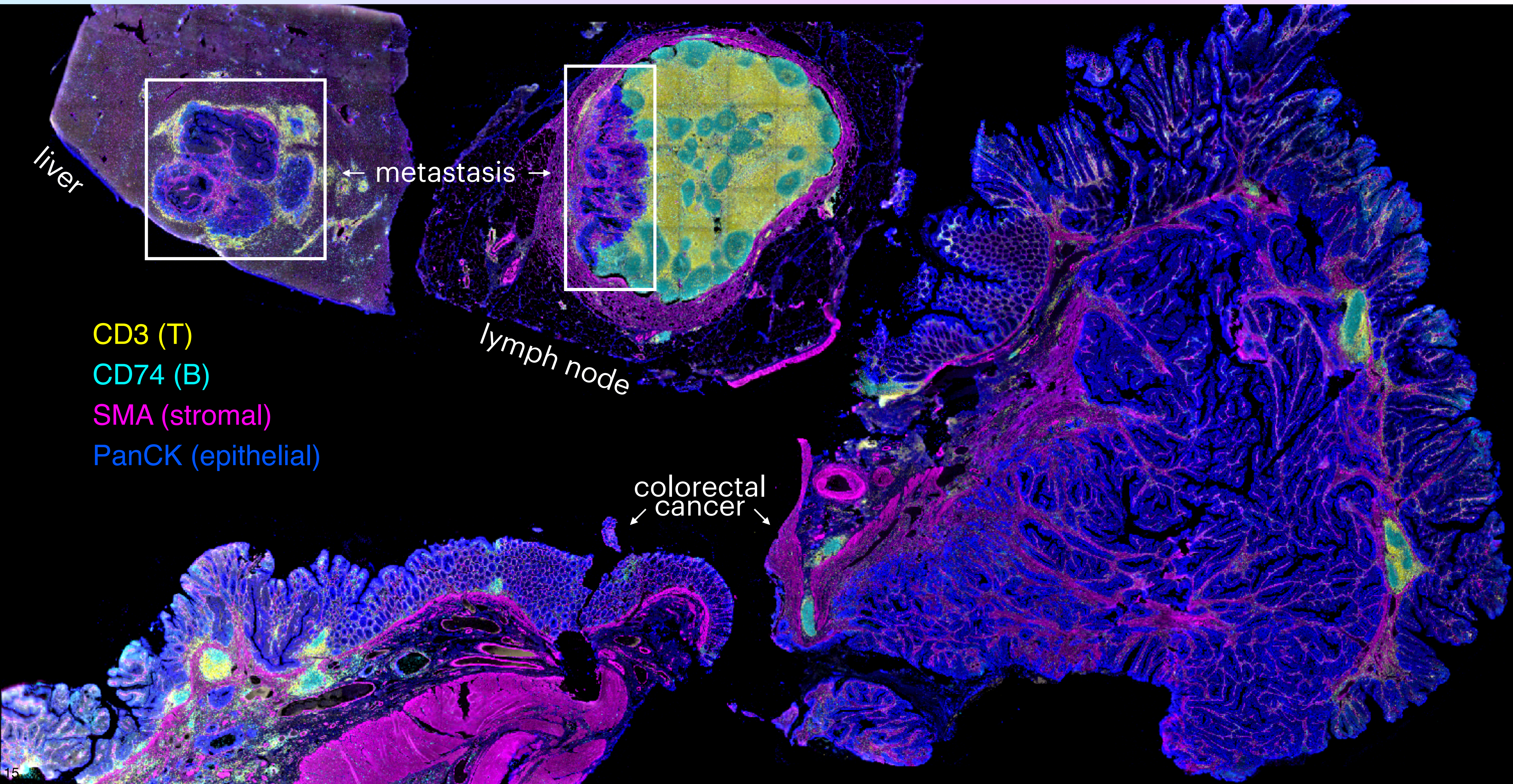


colorectal cancer



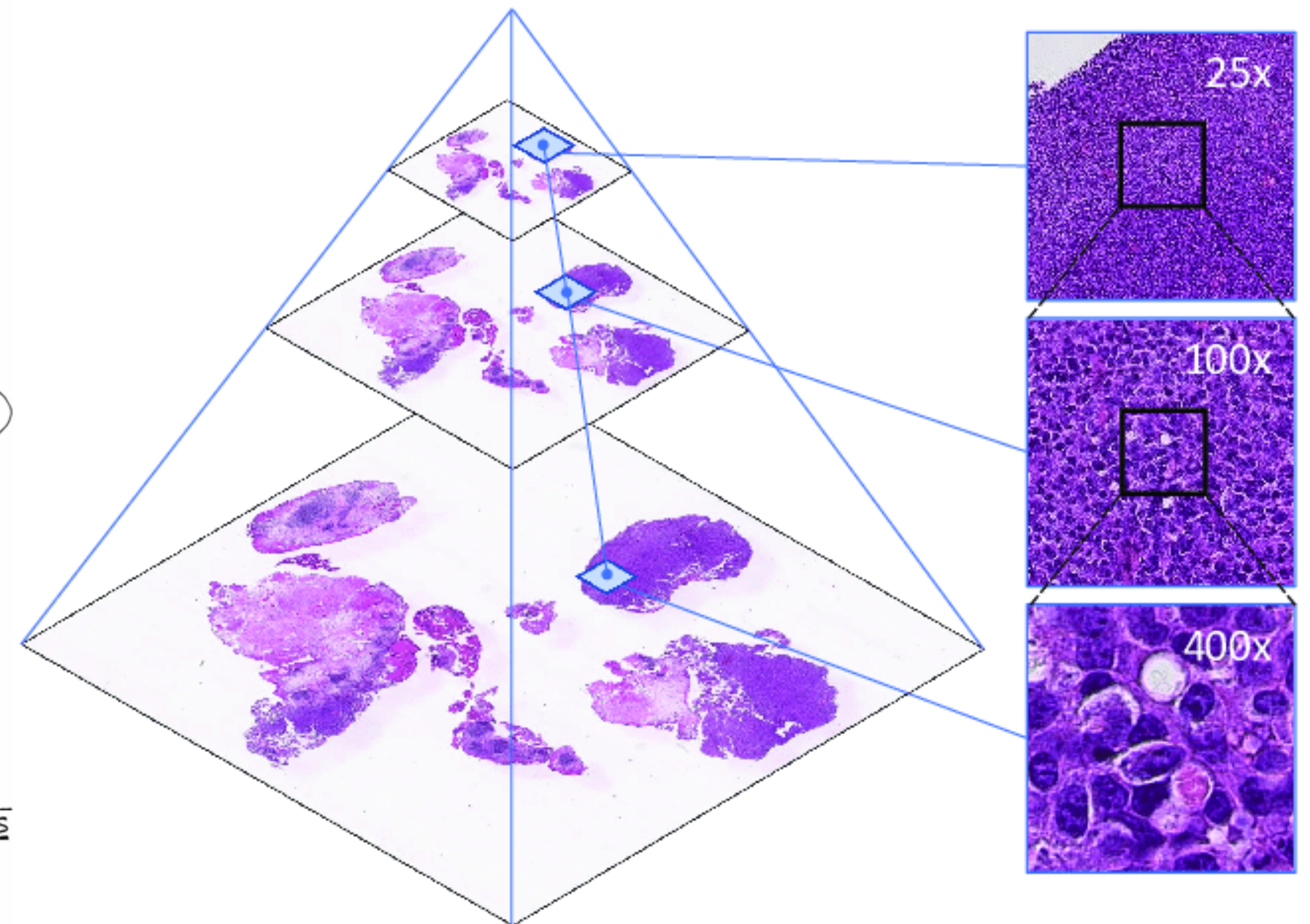
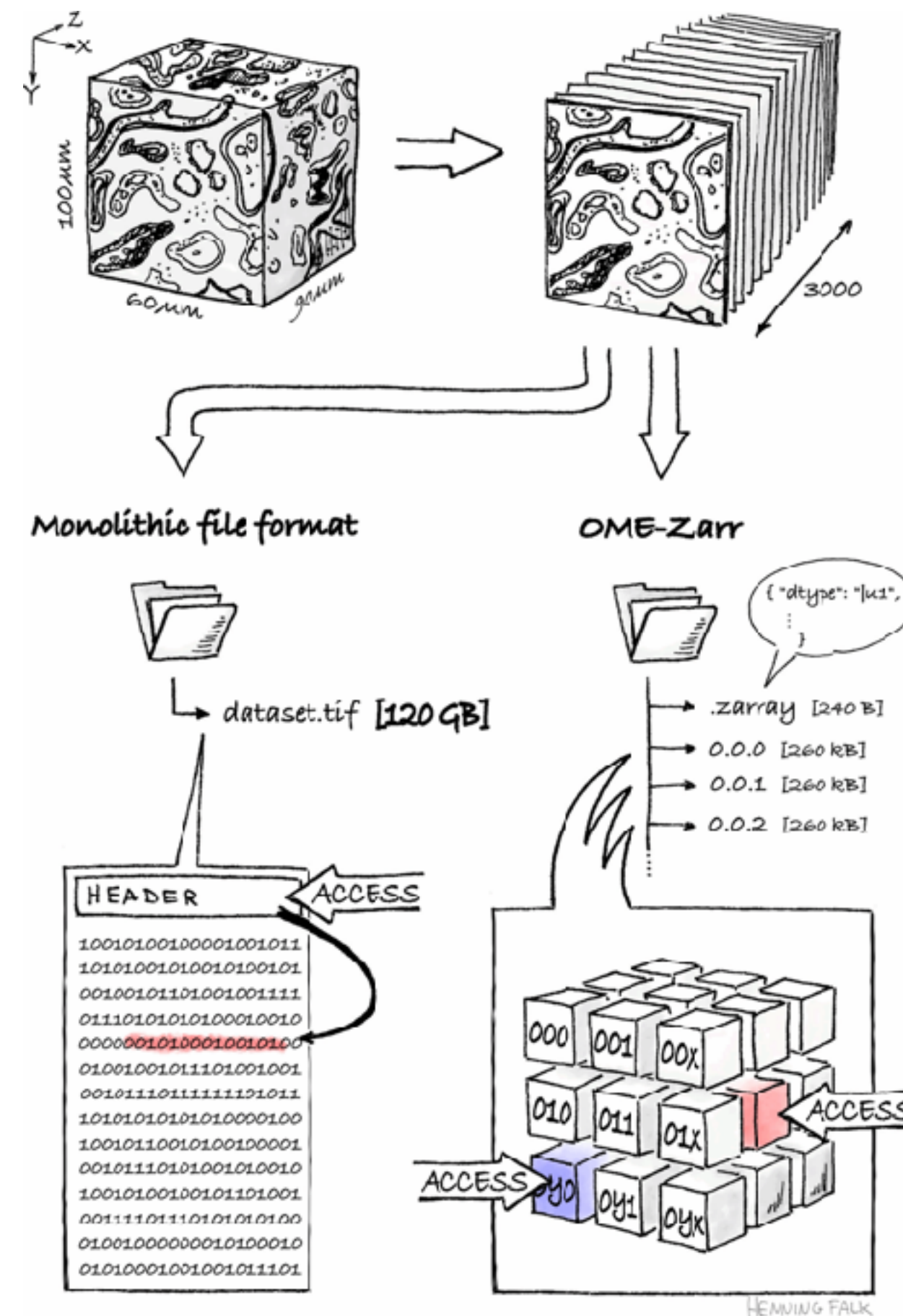
lymphovascular invasion

transcripts != proteins

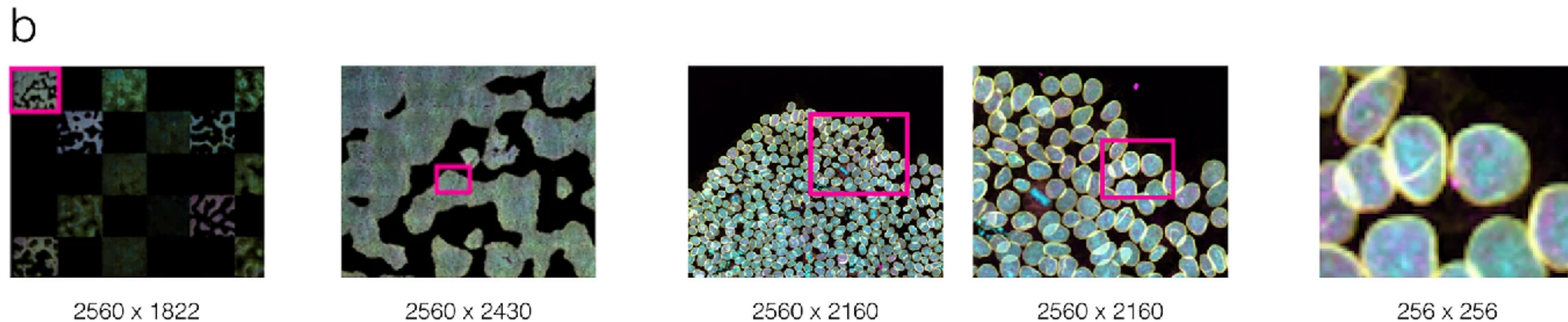
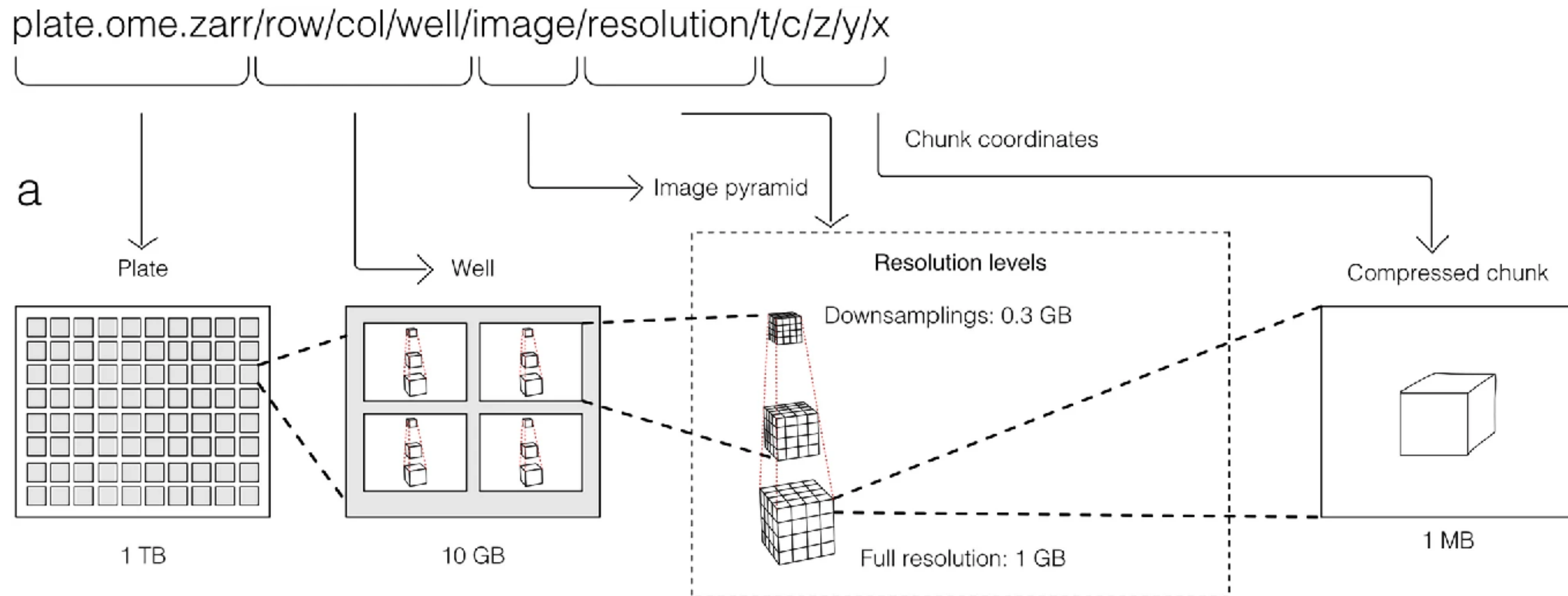


8TB images... over WIFI... from a café?

- **standardized**
on-disk format
& metadata
specifications
- **parallel & remote**
reading/writing
- **chunked**
(read what you need, but
as little more as possible)
- **multi-scale**
("pyramids")

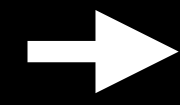


nested directories provide different levels of abstraction

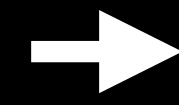


from tissue section to biological insight

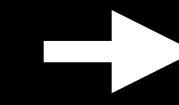
H&E staining



FOV placement



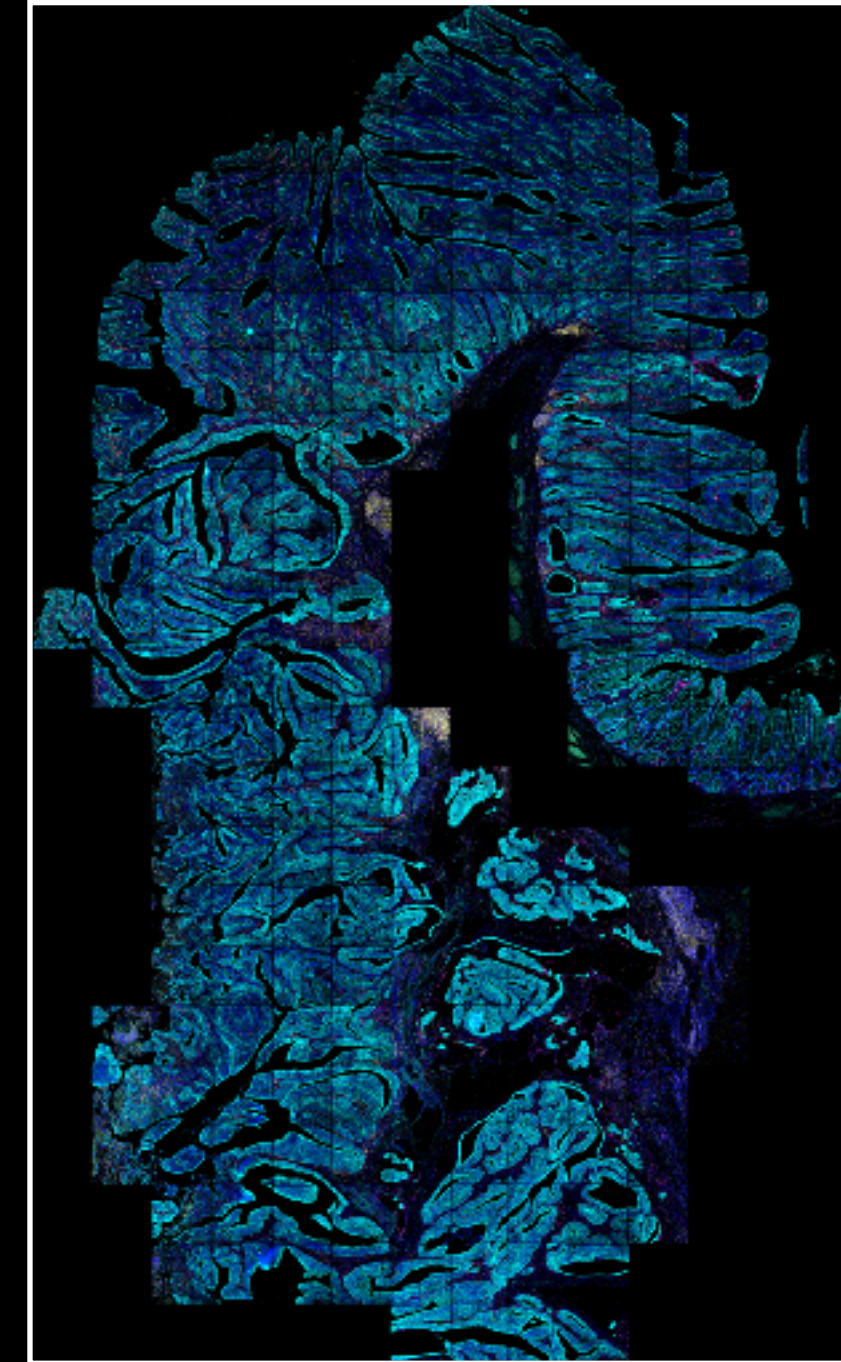
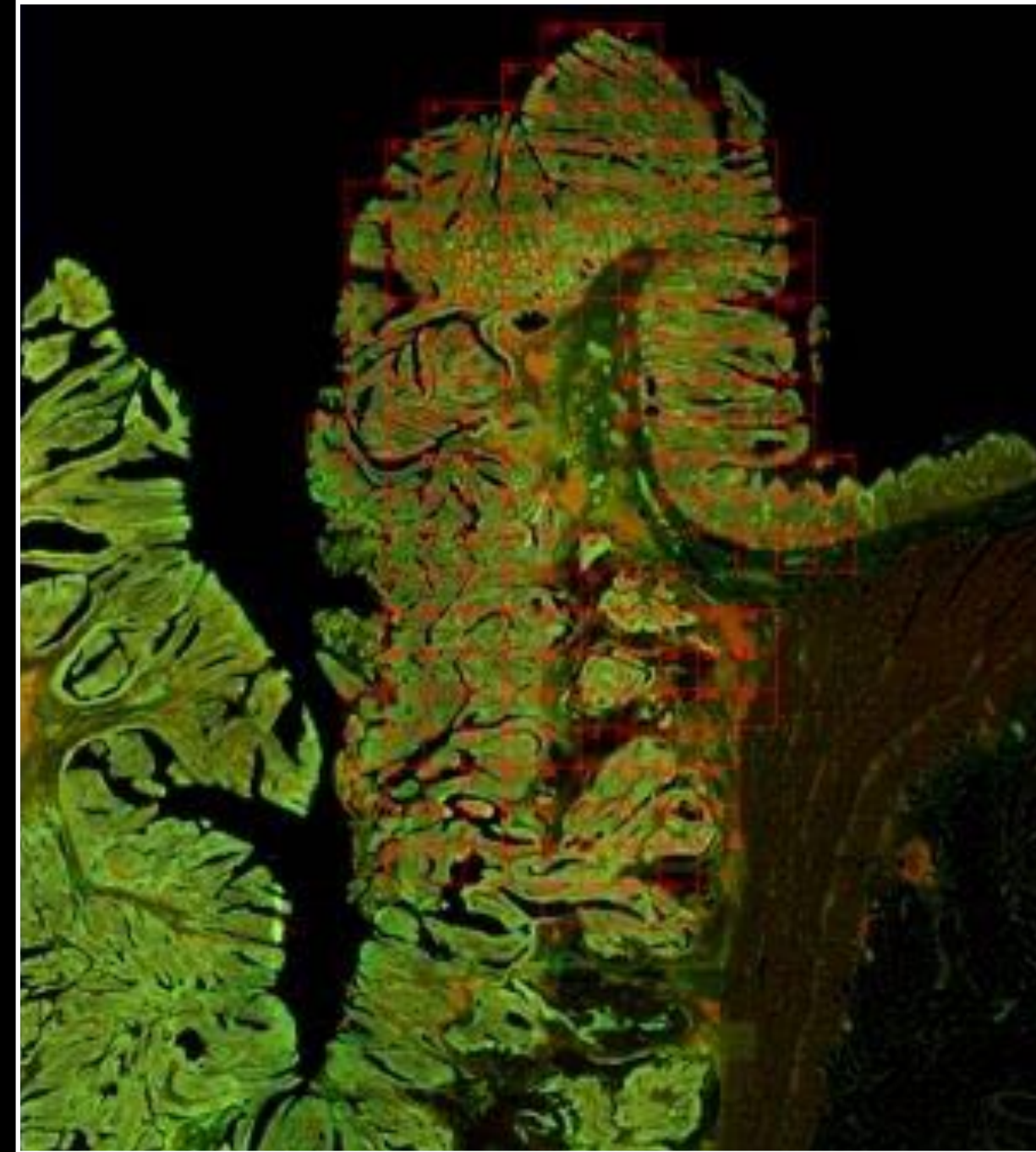
imaging



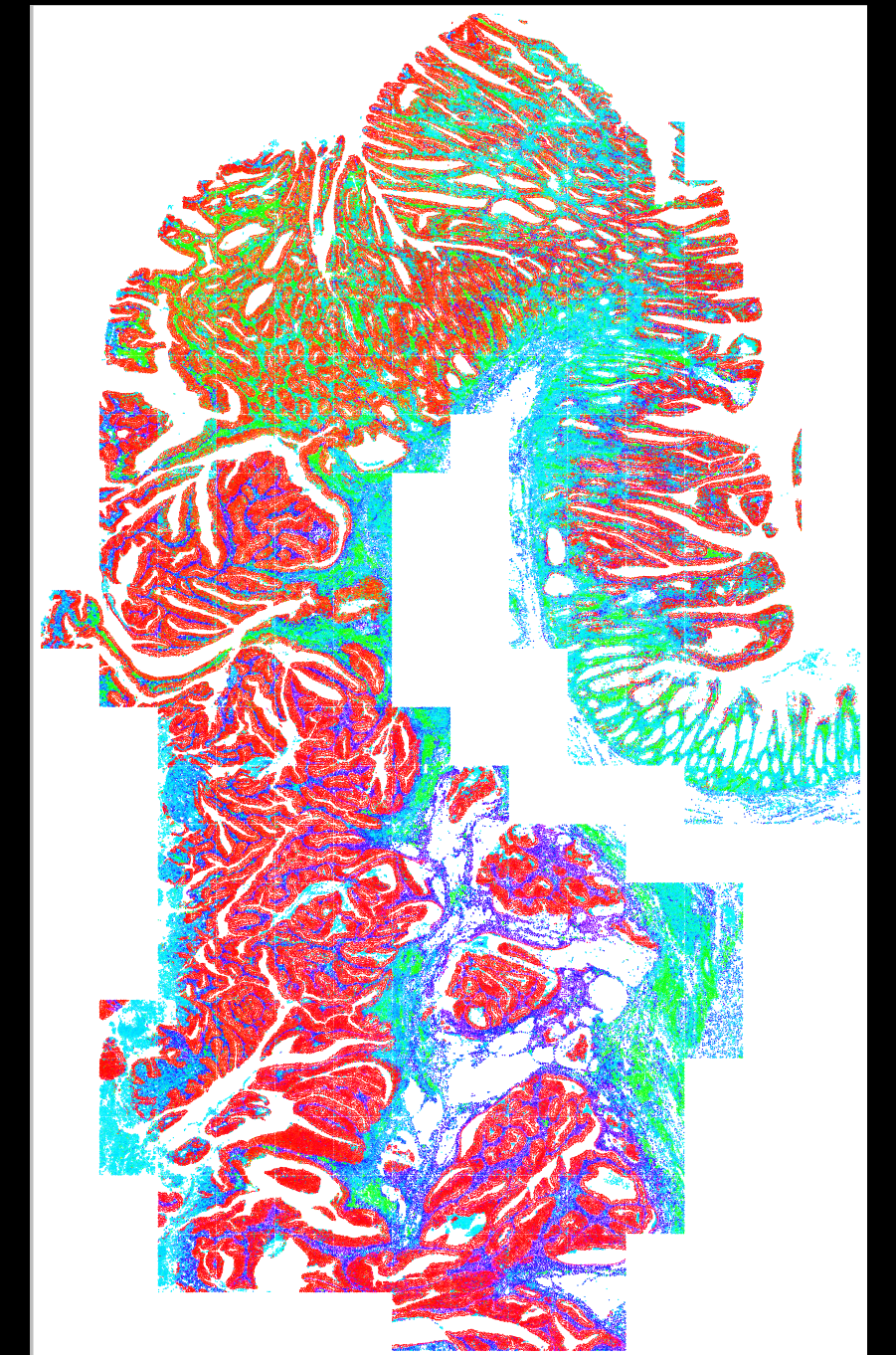
analysis



REF TVA CRC



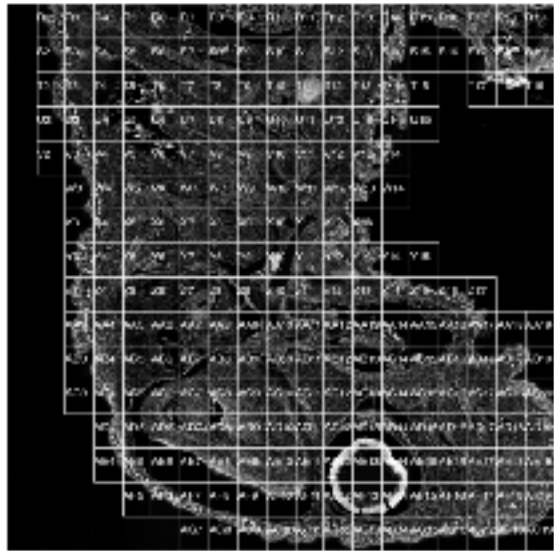
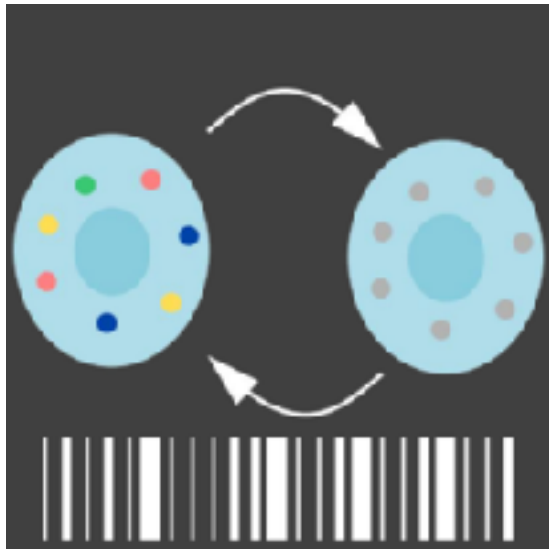
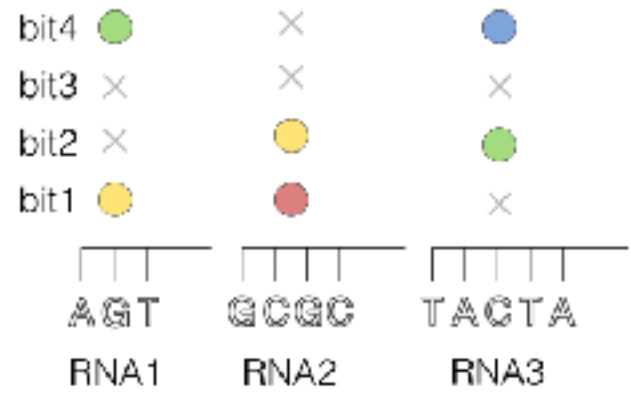
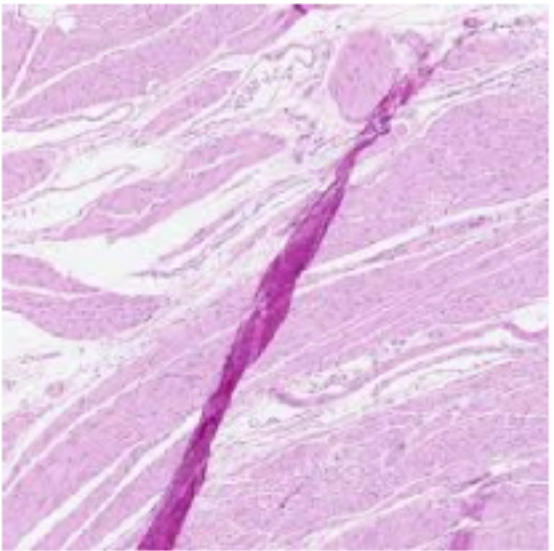
DAPI PanCK CD45 CD68



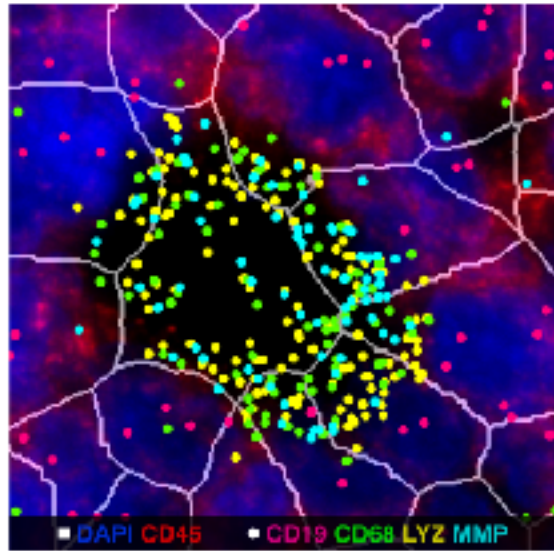
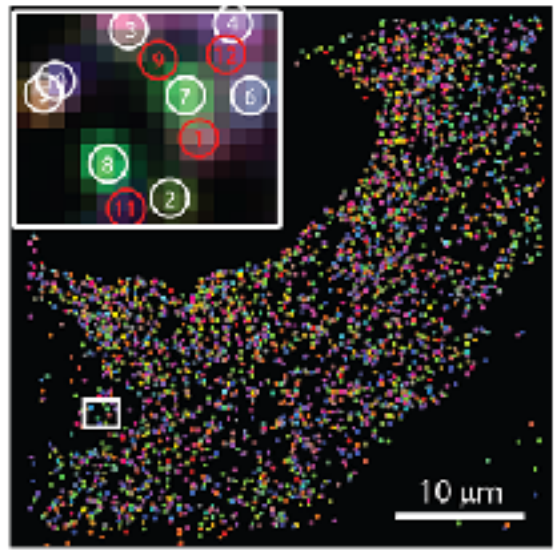
PC1 PC2 PC3

technical biases in imaging spatial transcriptomics

EXPERIMENTAL



COMPUTATIONAL



gene	cell	count
matrix		



potential
biases
affected
unit(s)

• biopsy • sectioning • preservation • slide mounting • histology artifacts	• probe length • probe set size • GC content • color barcodes • dye efficiency	• photo-bleaching • auto-fluorescence • cycling sequence • tissue degradation	• FOV stitching • z-compression	• optical crowding • spatial bleeding	• signal diffusion • cell morphology • auxiliary stainings
✗ ✗ ✓	✓ ✗ ✗	✓ ✗ ✓	✗ ✓ ✓	✓ ✗ ✗	✓ ✓ ✗

gene
cell
sample