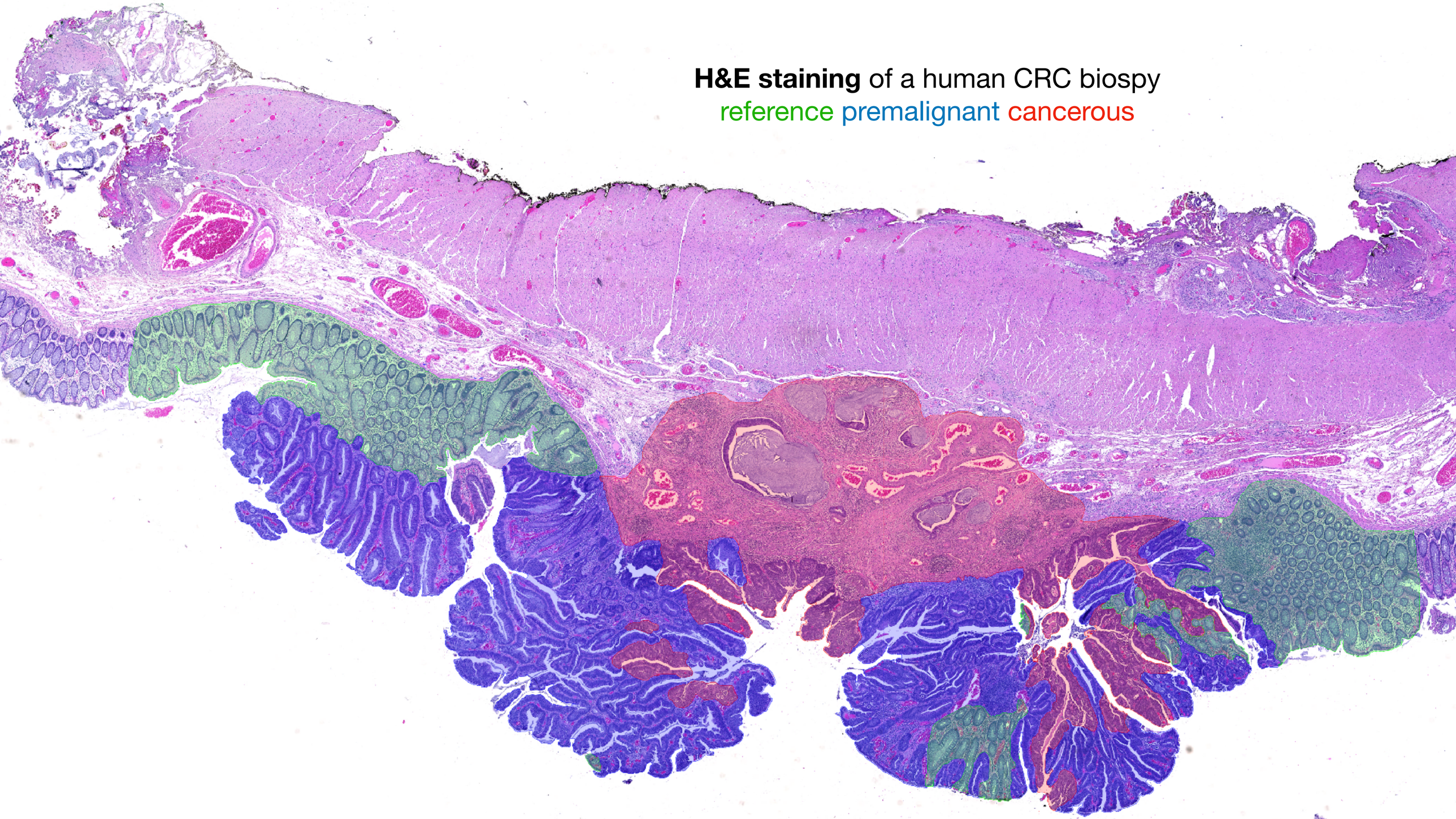
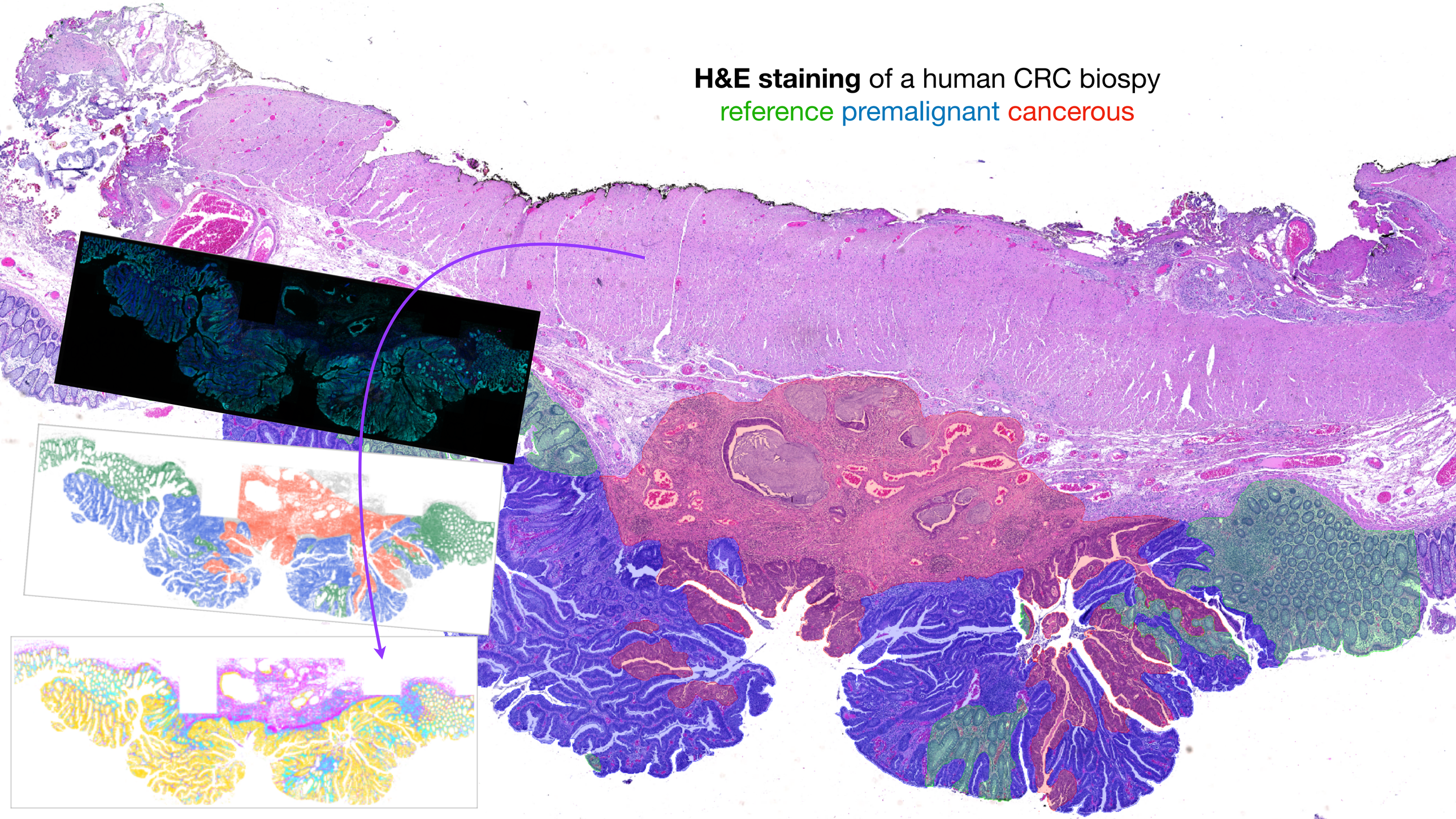
(pre)preprocessing of imaging-based spatial transcriptomics data

Swiss Institute of Bioinformatics
Spatial Omics Data Analysis

Helena L. Crowell — January 21st, 2025
in Lausanne, Switzerland


H&E staining of a human CRC biospy
reference premalignant cancerous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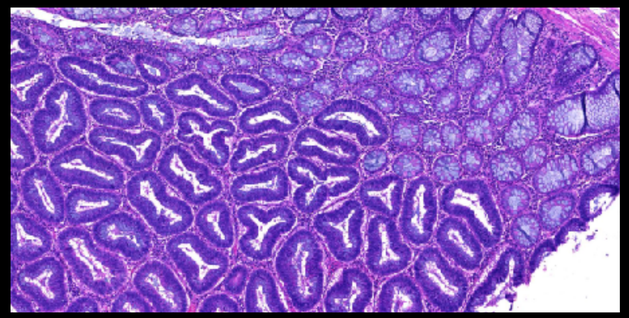
(sometimes)
stats.-/
AI-based

pathology ← H&E staining ← tissue



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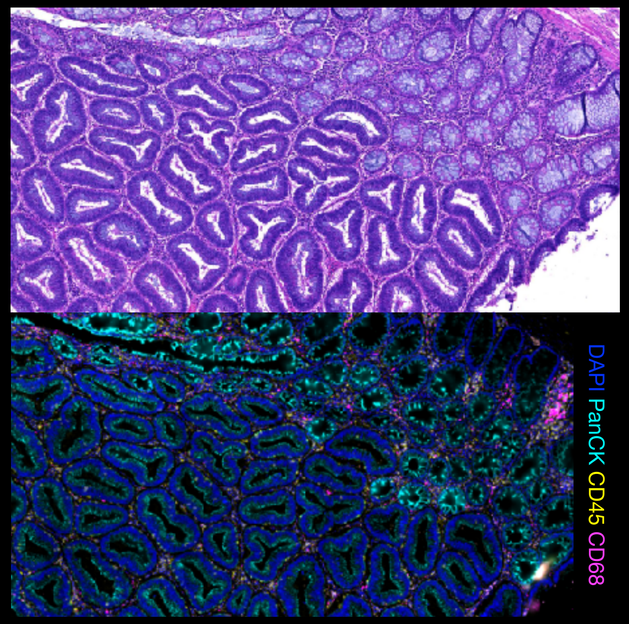
imaging



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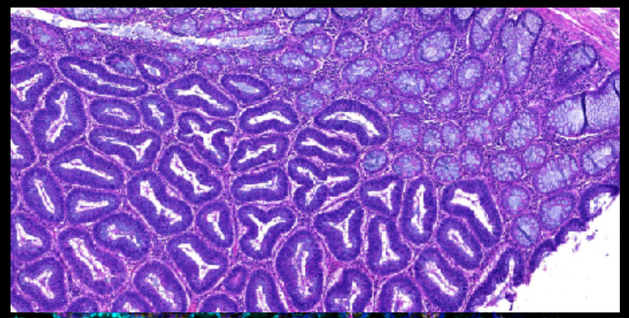
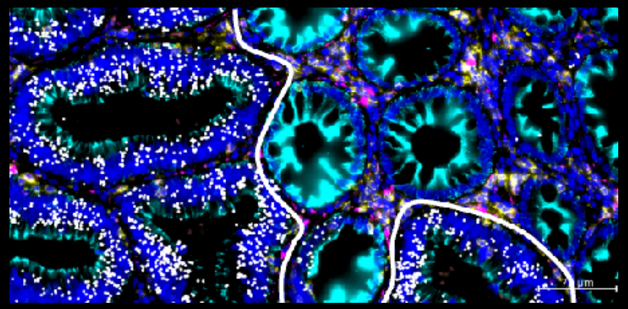
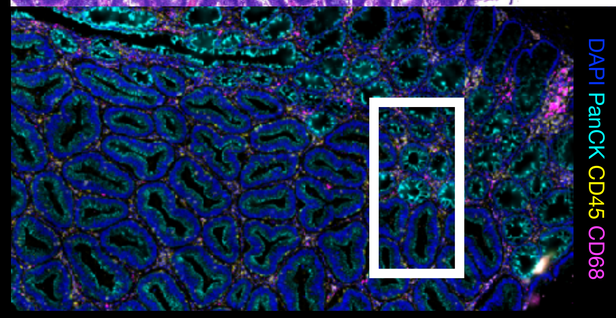
pathology ← H&E staining ← tissue → high-plex FISH → spot-calling → transcript molecules

tissue → imaging → IF staining







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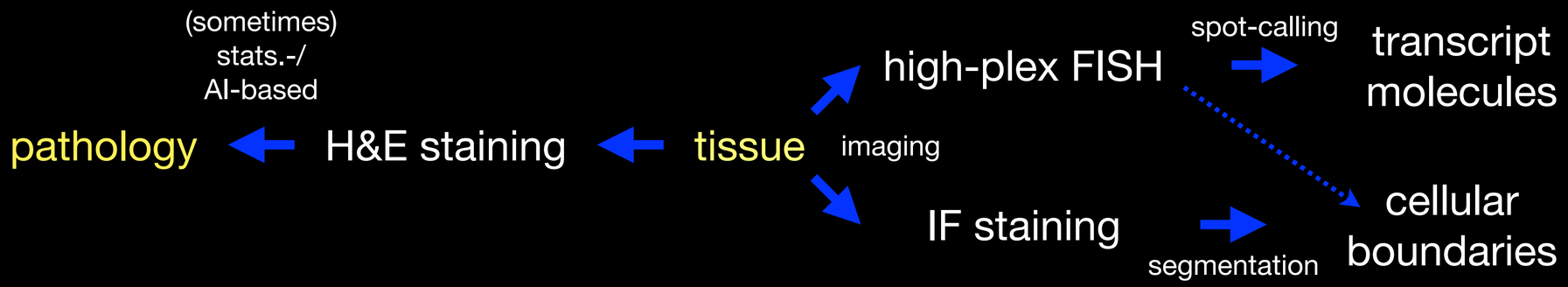

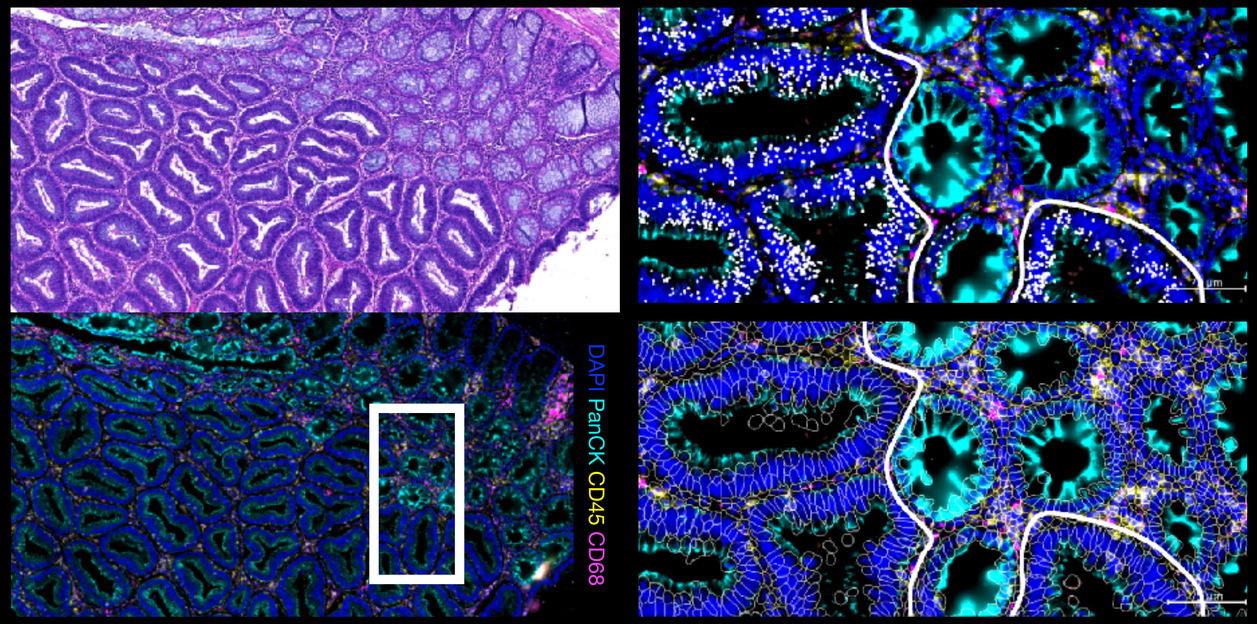

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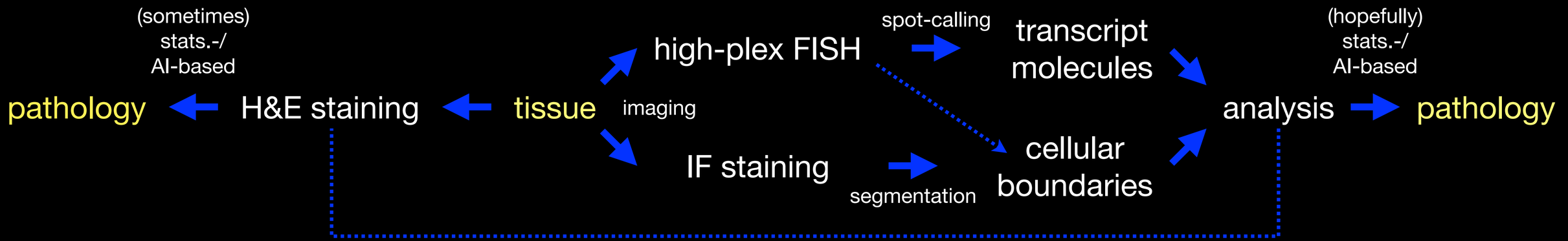

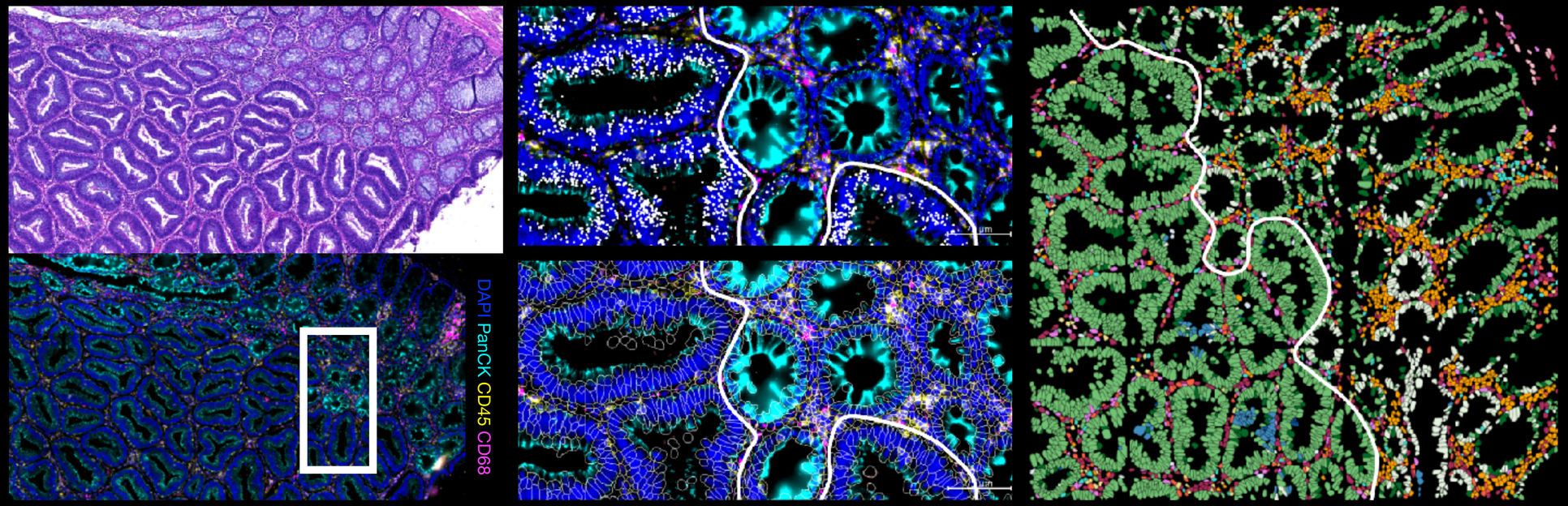

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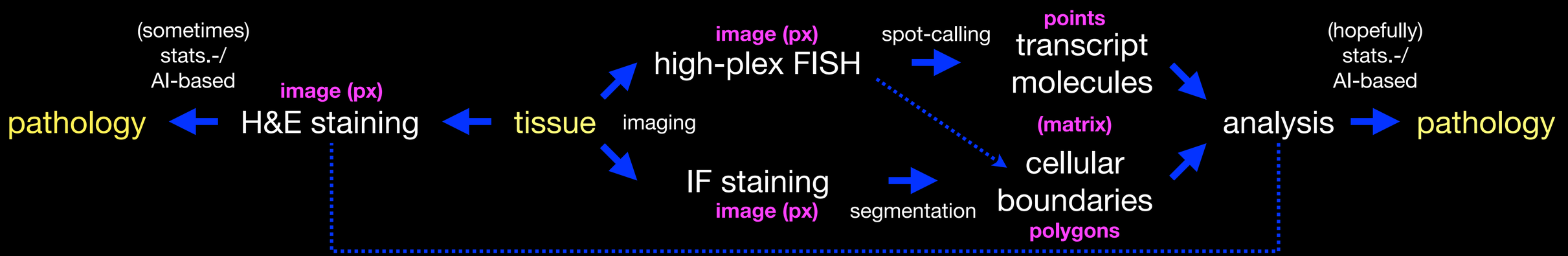

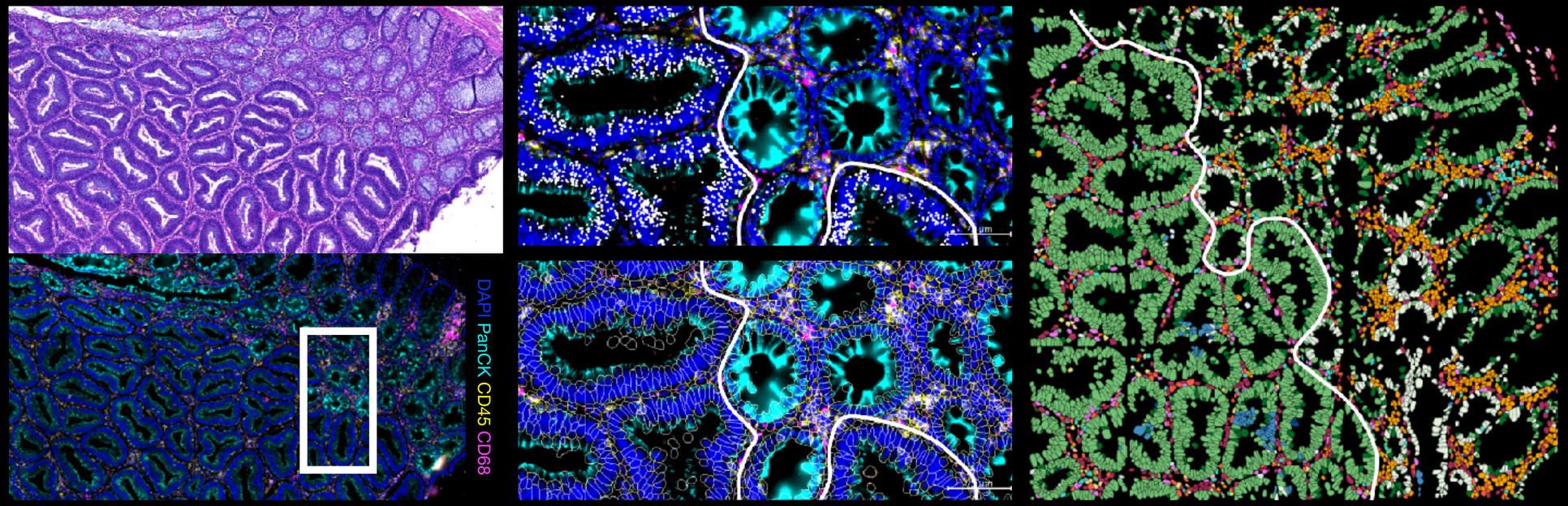

quality control for img-ST is ways from (sc)RNA-seq

high-throughput RNA sequencing

fragmentation, reverse transcription, mapping

↓

# of raw counts per transcript varies with transcript length, GC content, sequencing depth

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normalization strategies aim to minimize these effects & *“there’s awareness that misinterpretation of results where biological & technical effects are correlated”*

Martin *et al.* (2024), *bioRxiv 2024.12.04.626766*

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tissue preparation, chemistry, imaging

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tissue damage/detachment, image/transcript loss, varying detection across space & experiments

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quality control for img-ST is ways from (sc)RNA-seq

DISCLAIMER: We don't really know what's best (yet) —
I'll try to summarize some recent ideas, and personal pains.

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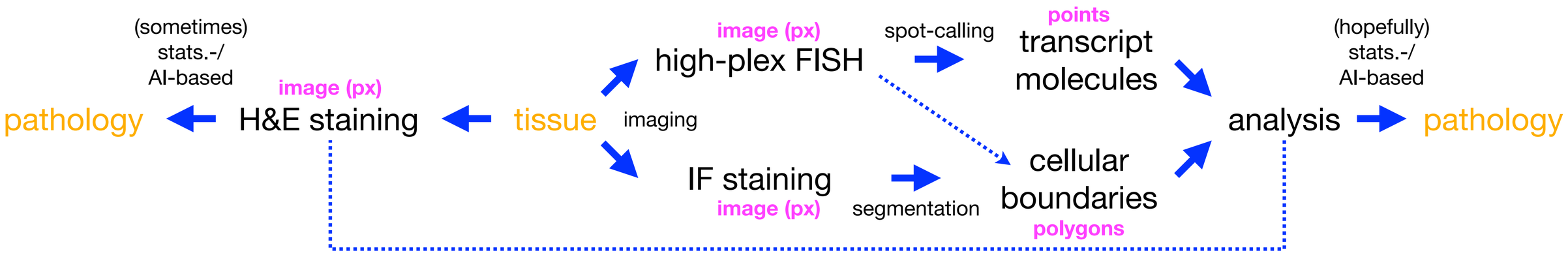

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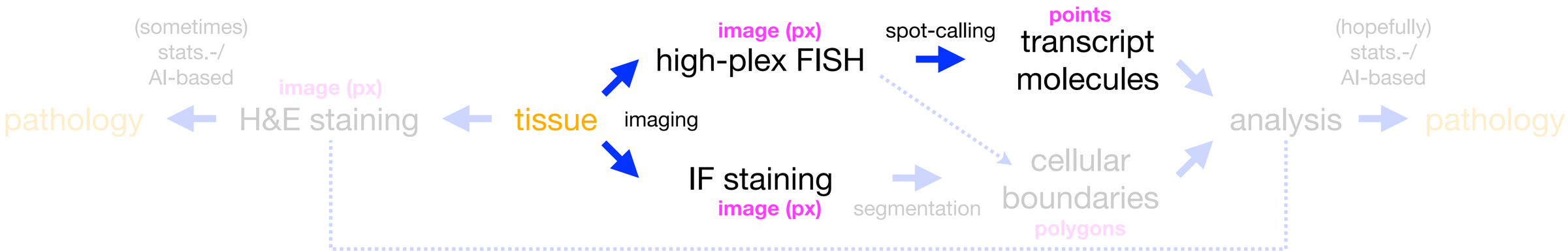

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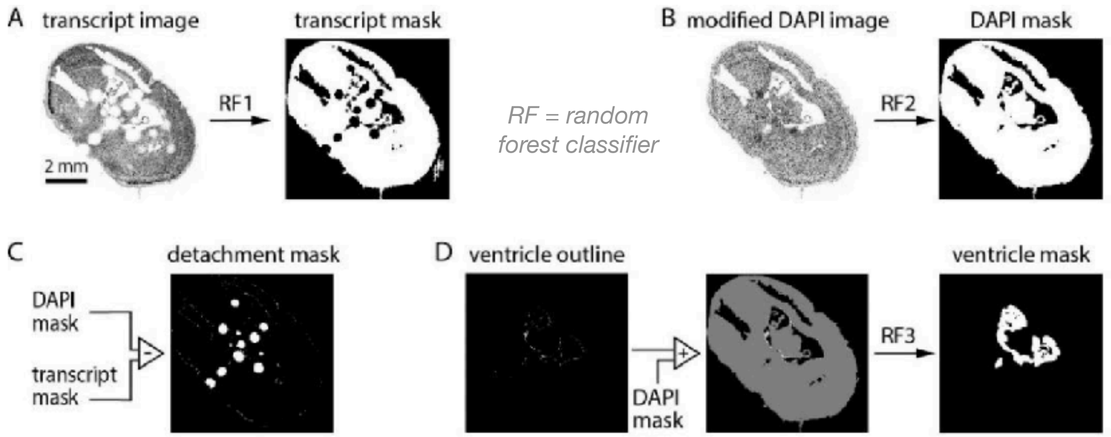

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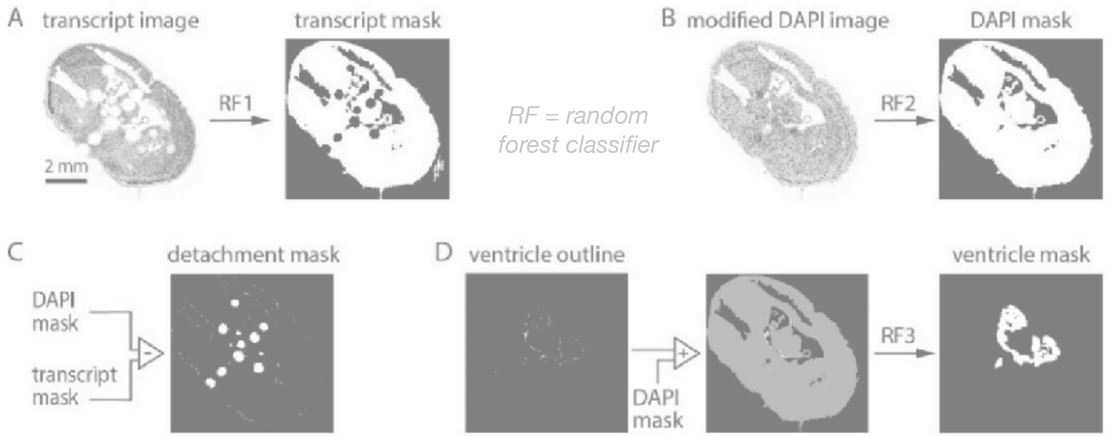
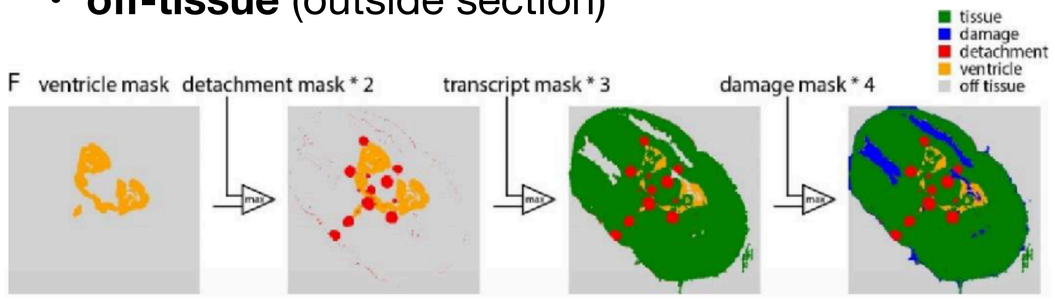


- each location is classified into one of **five categories**
  - **tissue** within image volumen
  - **detachment** (tissue not imaged)
  - **ventricle** (no tissue but no loss)
  - **damage** (no tissue due to loss)
  - **off-tissue** (outside section)



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MerQuaCo proposes a pixel classifier & **quality metrics**

perfusion rate

- log files can reveal inconsitencies (e.g., blockage) of volume per time during solution exchange

data loss

- iterative comparison of transcript counts between neighboring fields of view (FOVs)

detection efficiency

- across section: periodicity,
  through section: p6/p0 ratio

transcript density

- should vary across section, but little between sections of comparable quality

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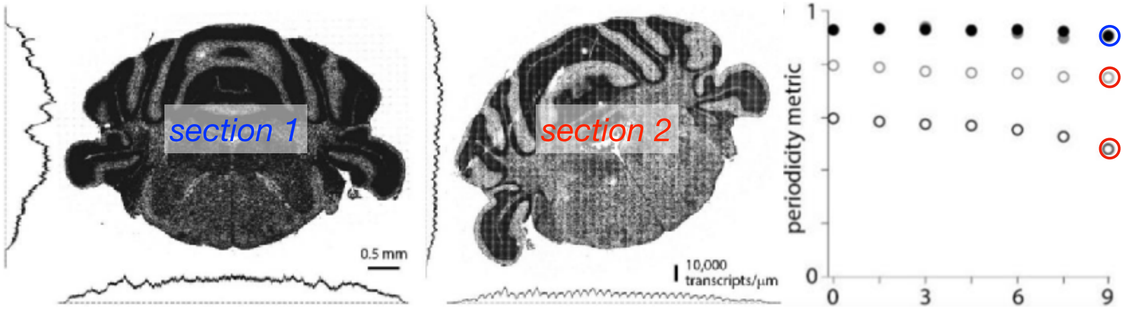

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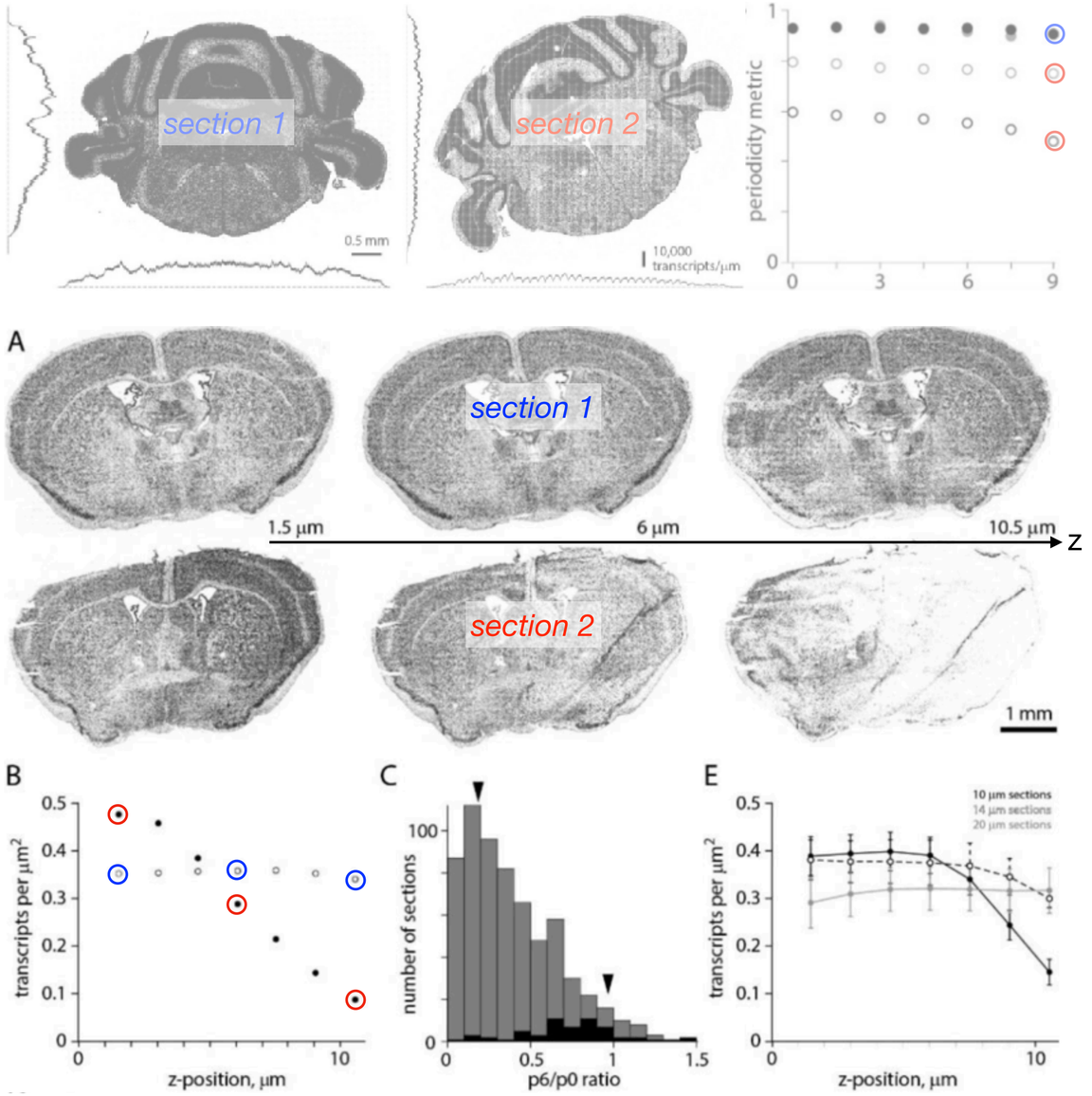

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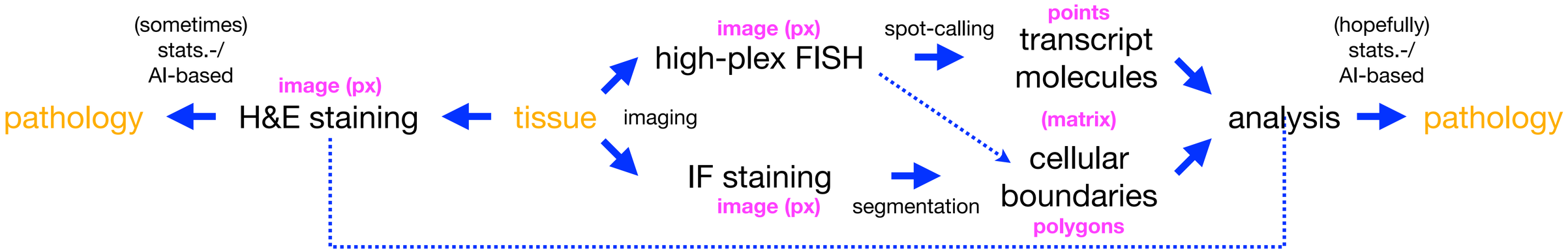
for (most) analyses, we need to go **from images to counts**



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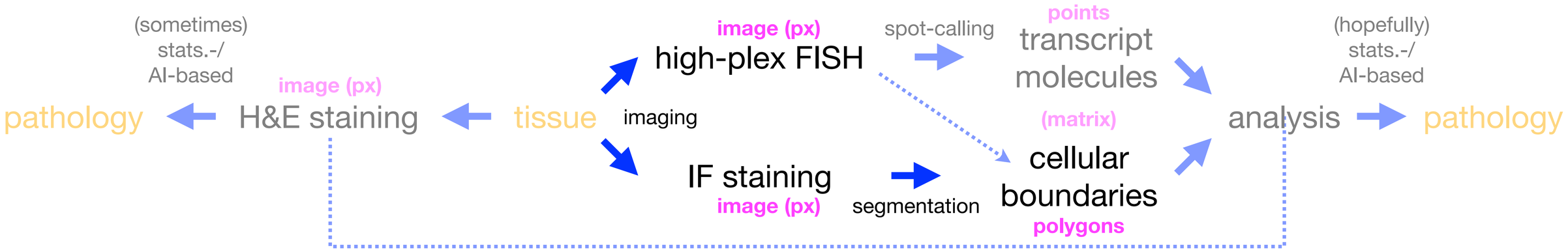

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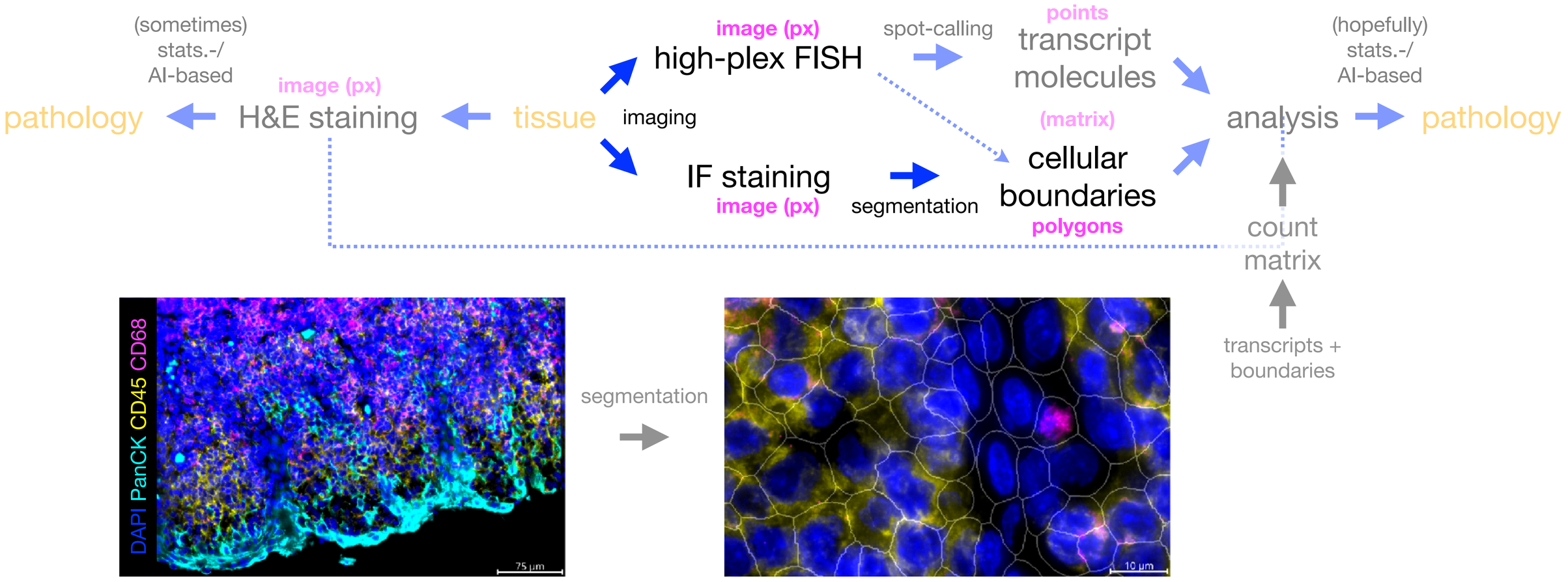

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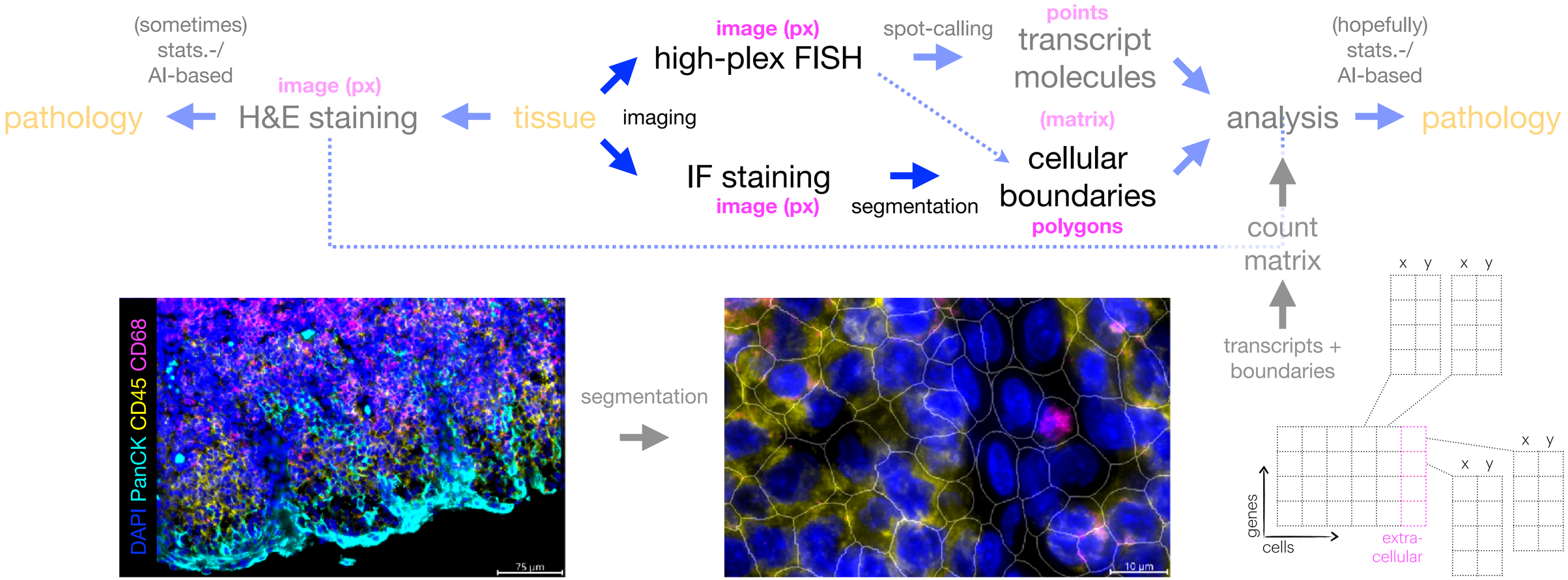
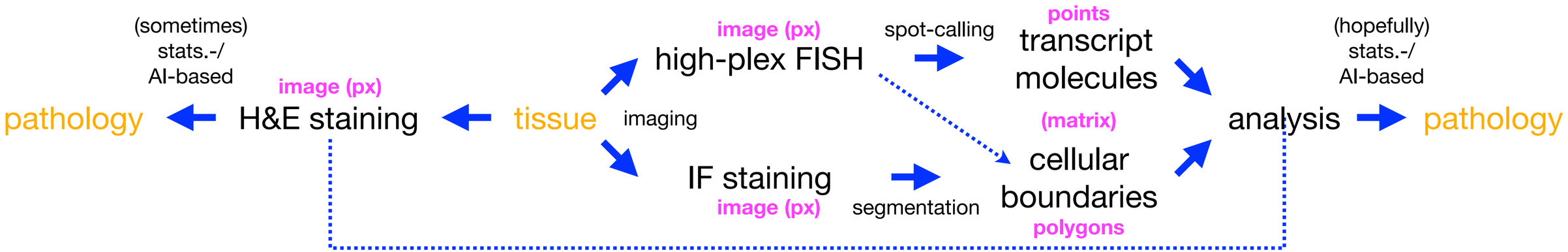


beware standard QC metrics — counts relate area AND biology



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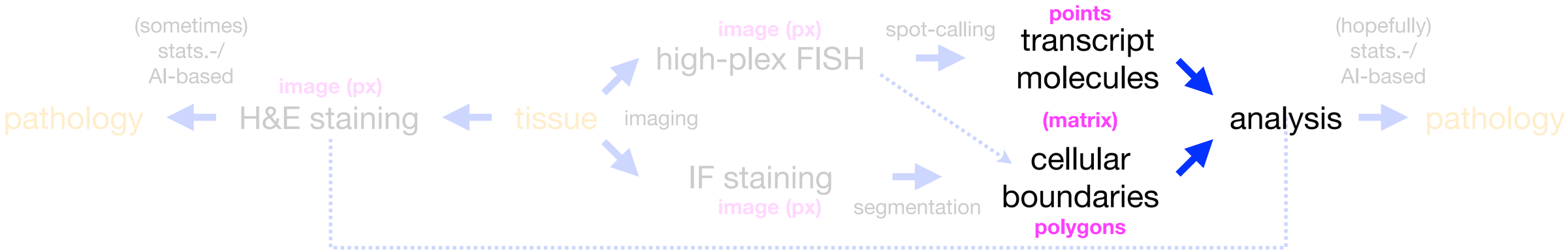

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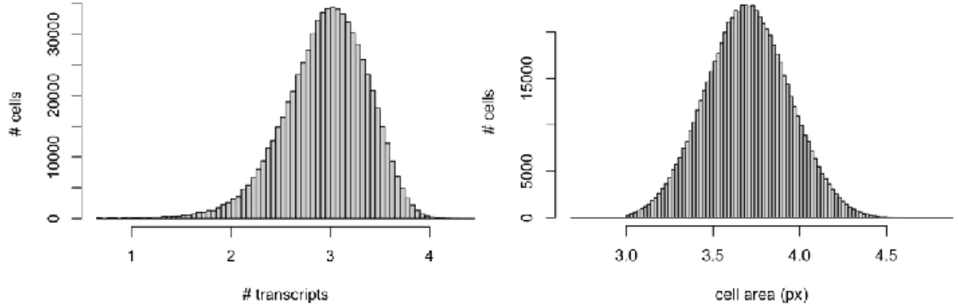

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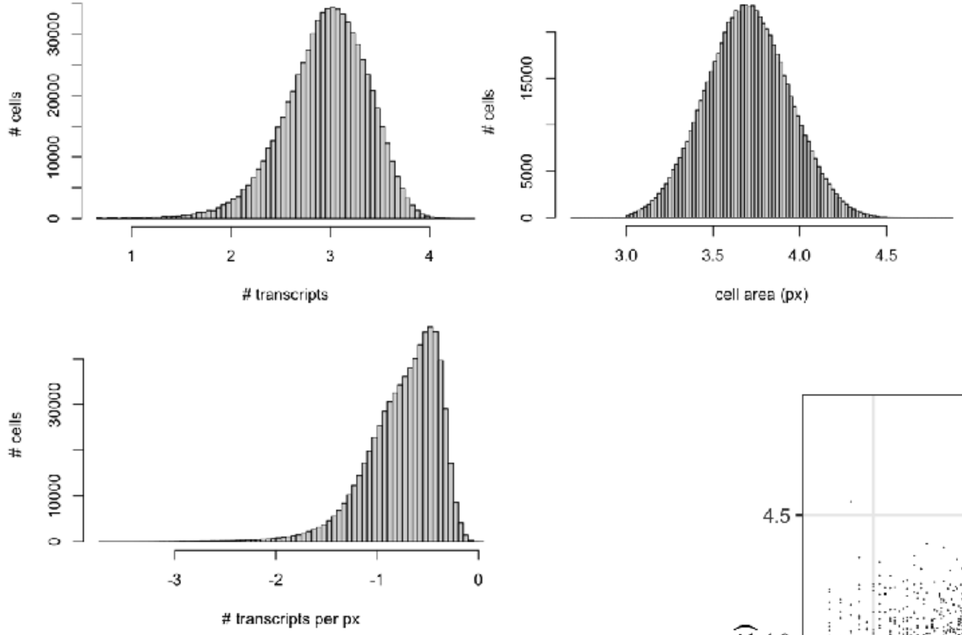

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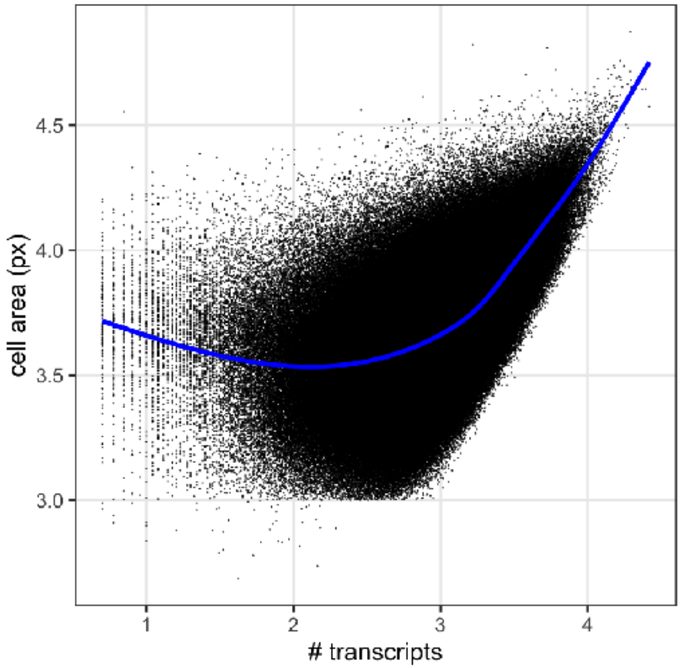

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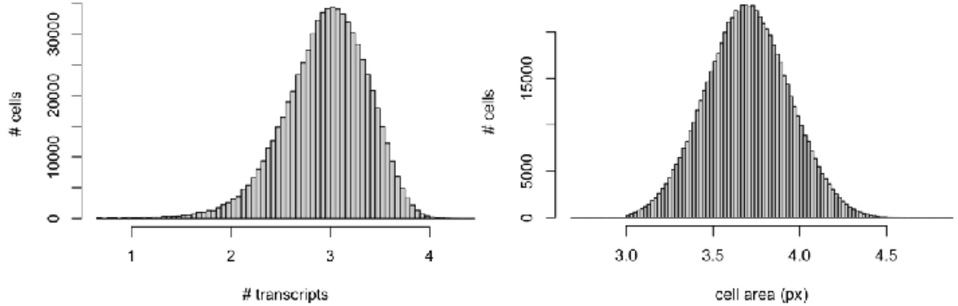
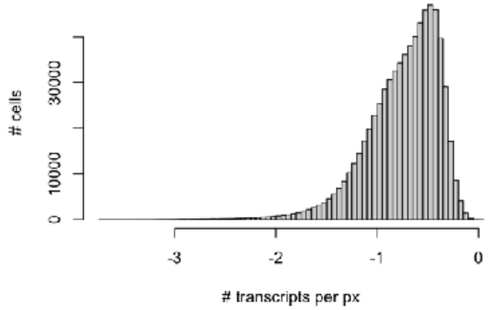
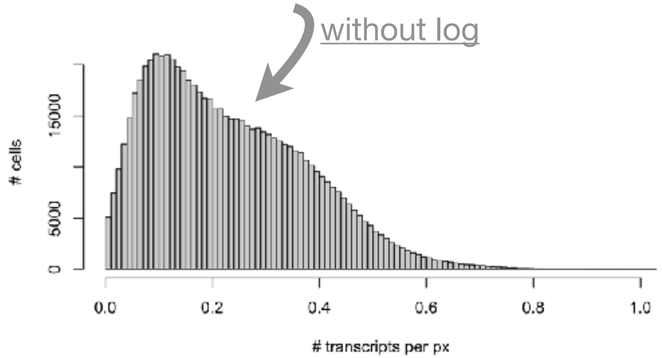




without log



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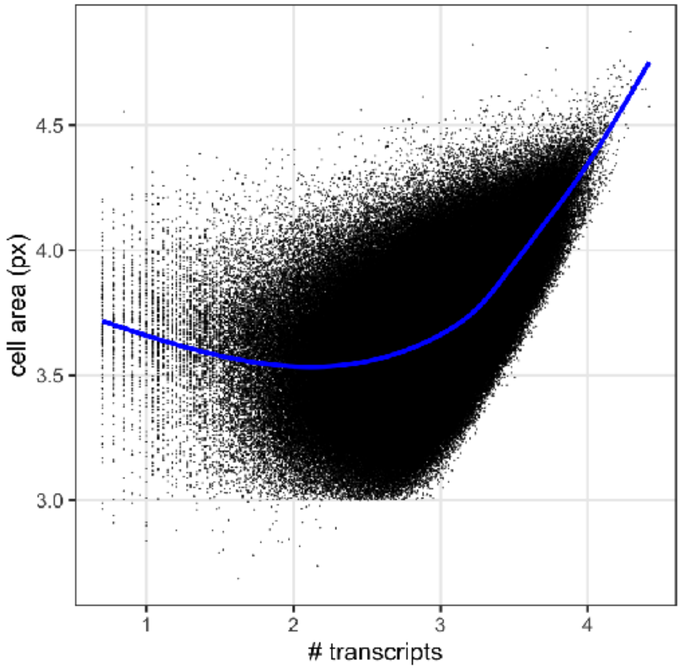

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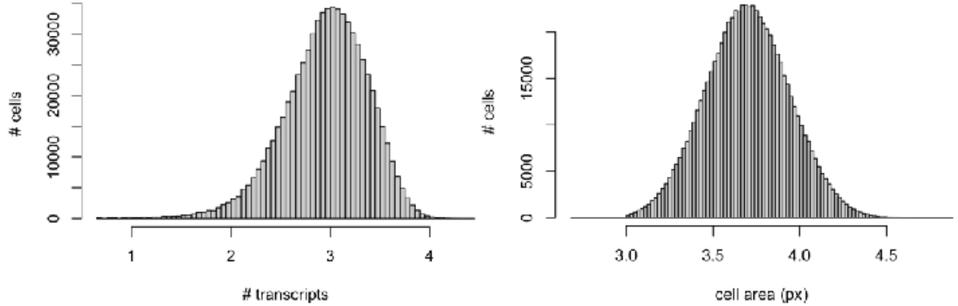

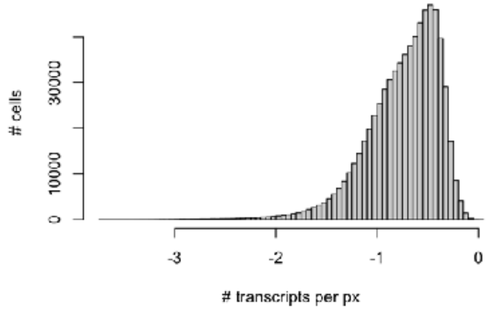

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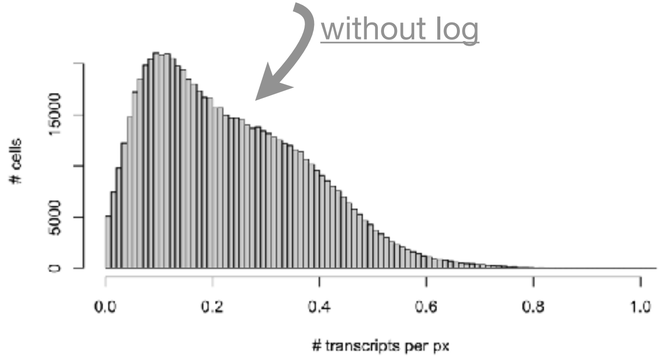

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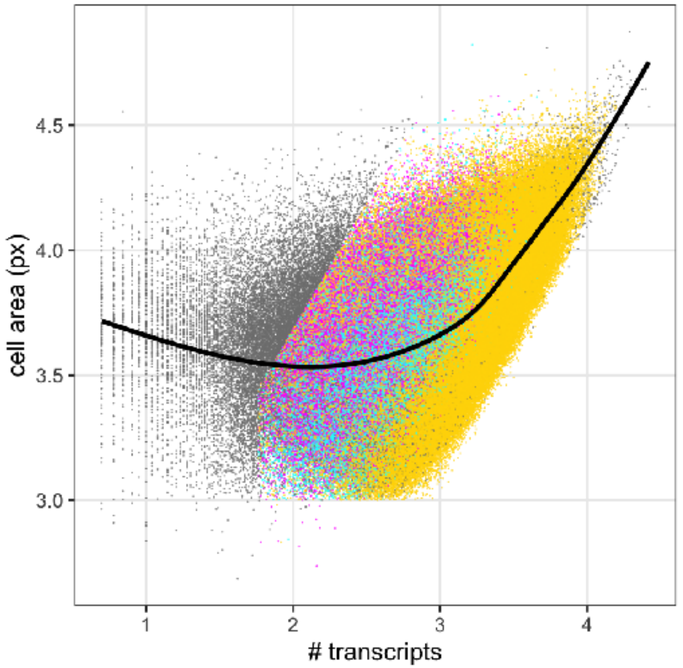
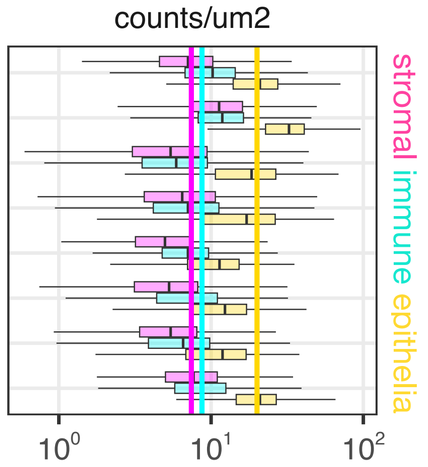
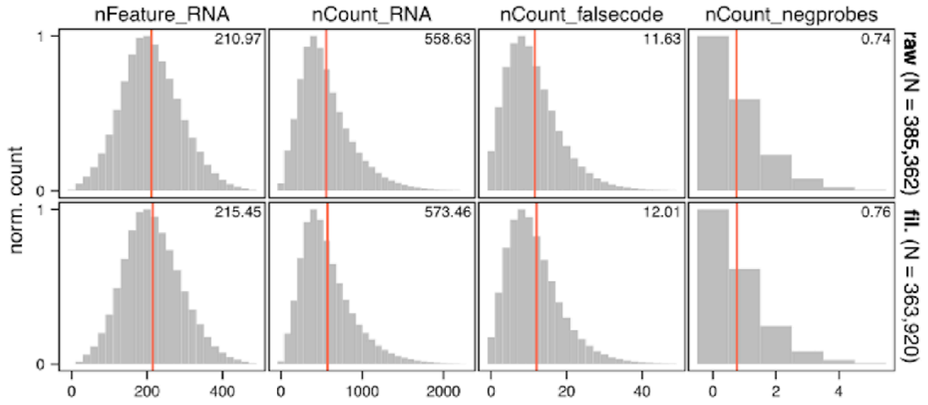


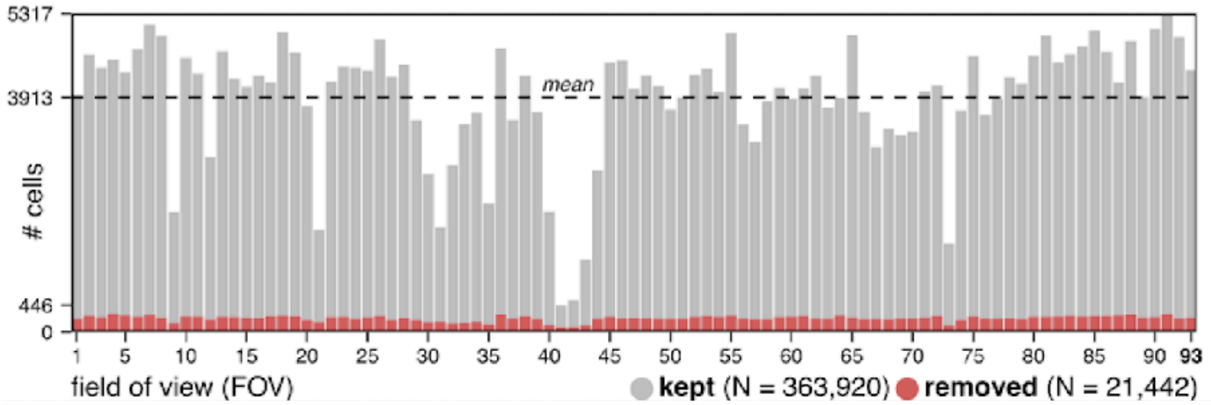


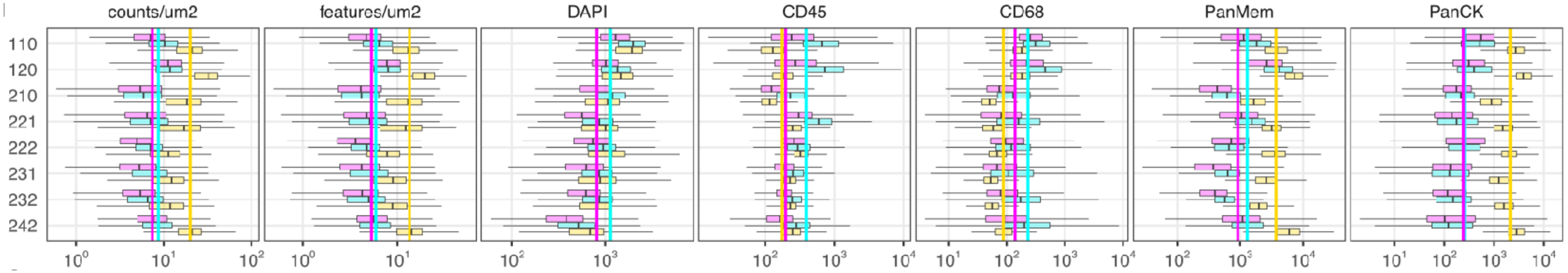
but there's more we could be looking at… some **QC examples**

standard QC metrics



 # cells across FOVs





RNA per area & IF markers

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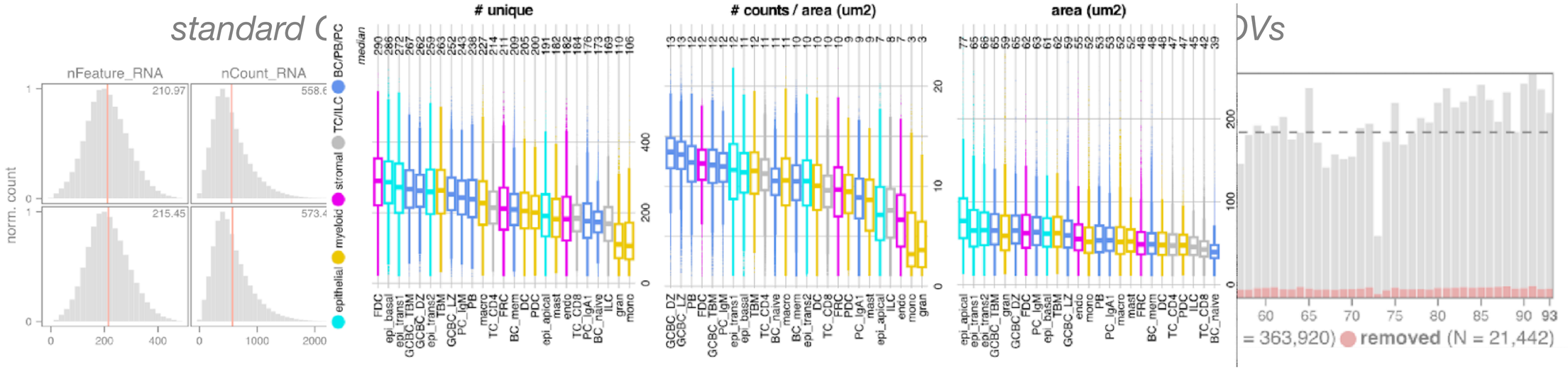
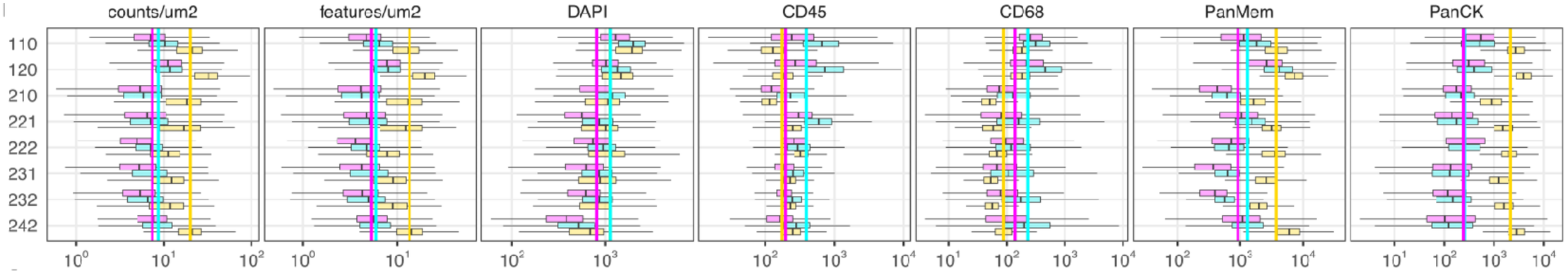
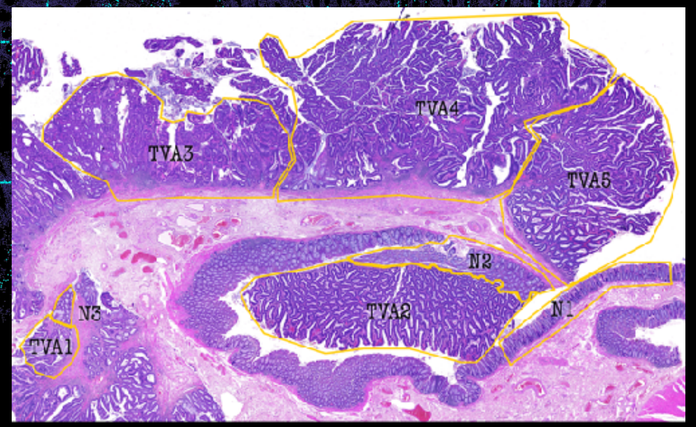




RNA per area & IF markers

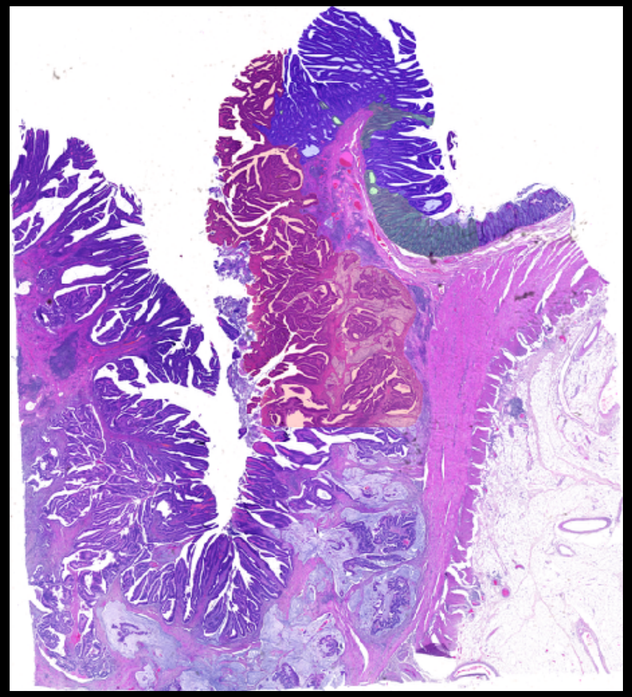
spatial organization of excluded cells might indicate a **bias against specific types** (but it depends!)

retained
excluded



img-ST data stem from serial imaging of **fields of view (FOVs)**

H&E staining

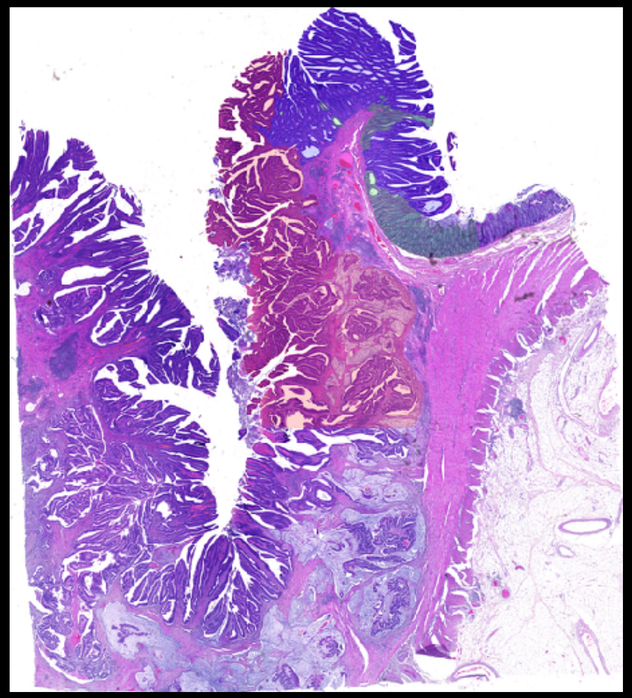


REF TVA CRC

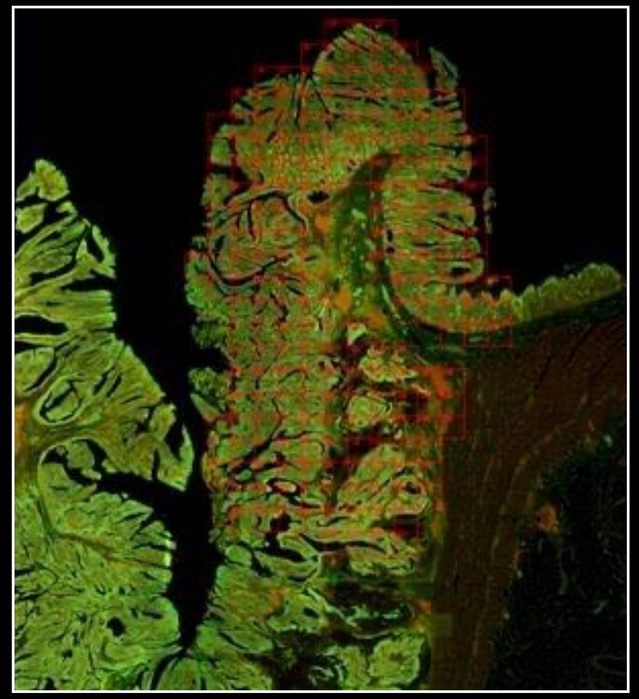
Crowell *et al.* Tejpar & Heyne (in prep.)

img-ST data stem from serial imaging of **fields of view (FOVs)**

H&E staining → FOV placement



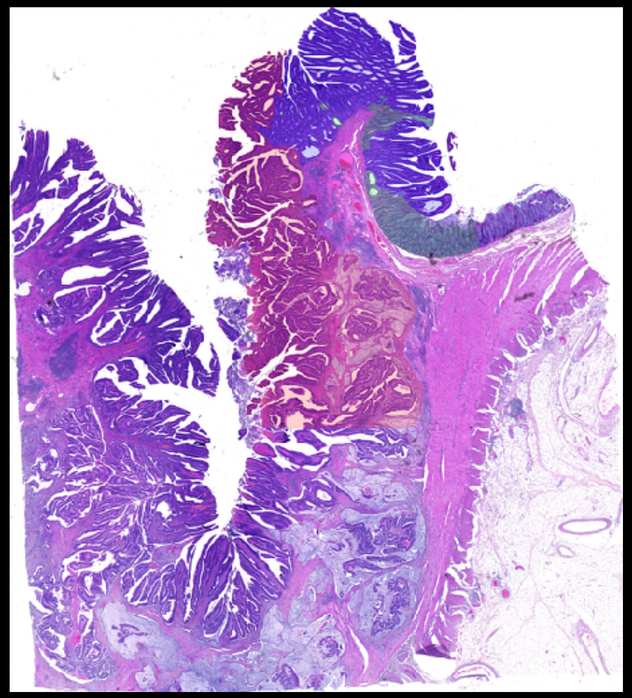
REF TVA CRC



Crowell *et al.* Tejpar & Heyne (in prep.)

img-ST data stem from serial imaging of **fields of view (FOVs)**

H&E staining → FOV placement → imaging



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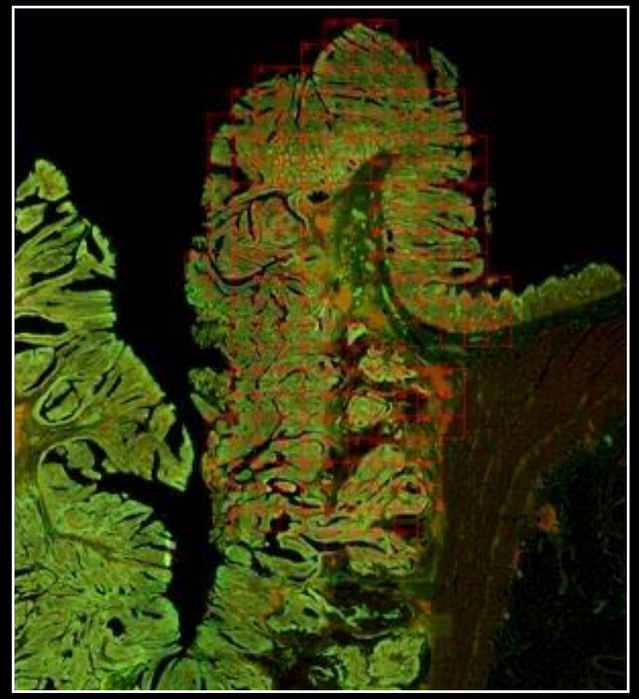
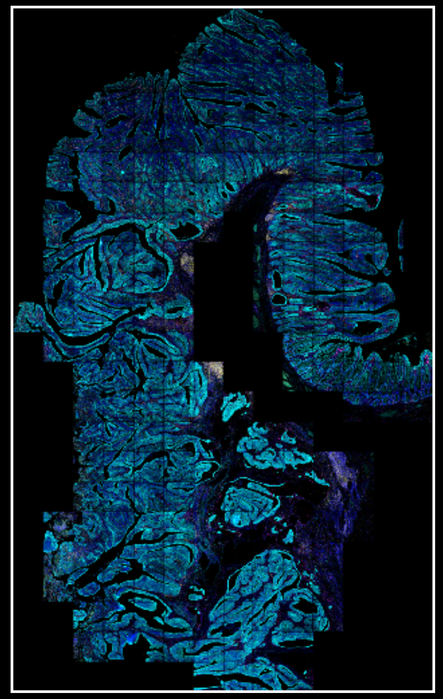


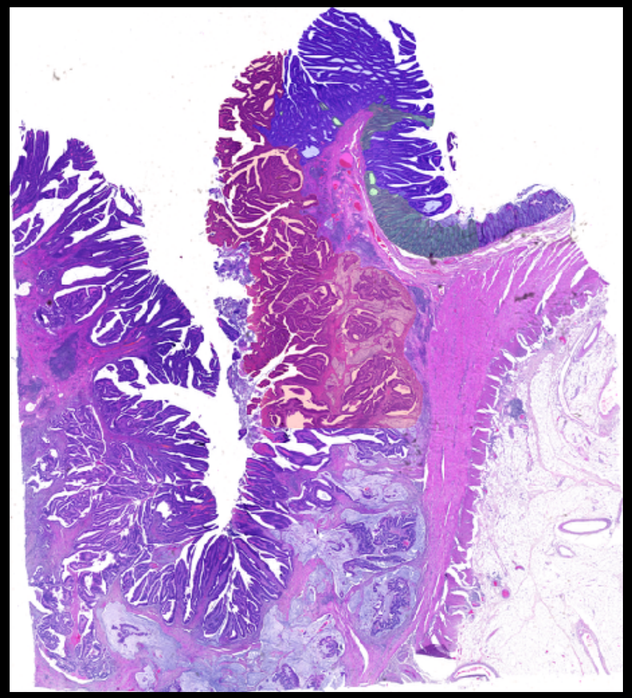


DAPI PanCK CD45 CD68

Crowell *et al.* Tejpar & Heyne (in prep.)

img-ST data stem from serial imaging of **fields of view (FOVs)**

H&E staining → FOV placement → imaging → analysis



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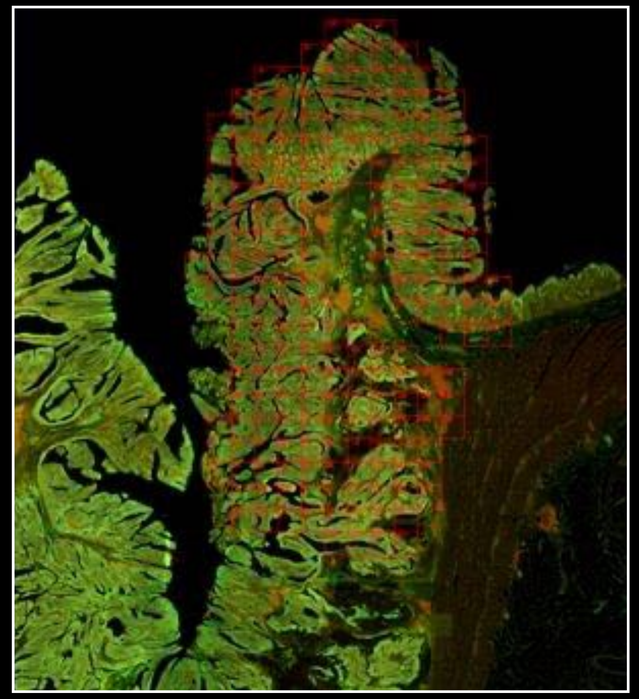
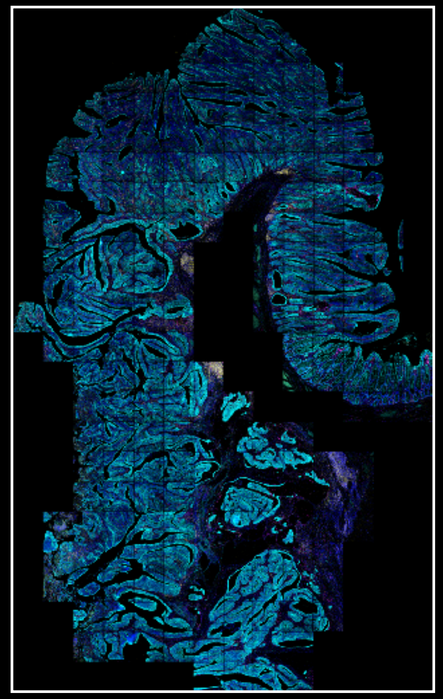
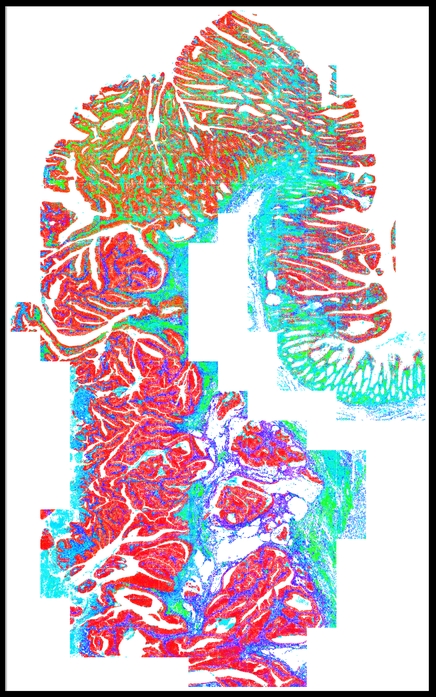




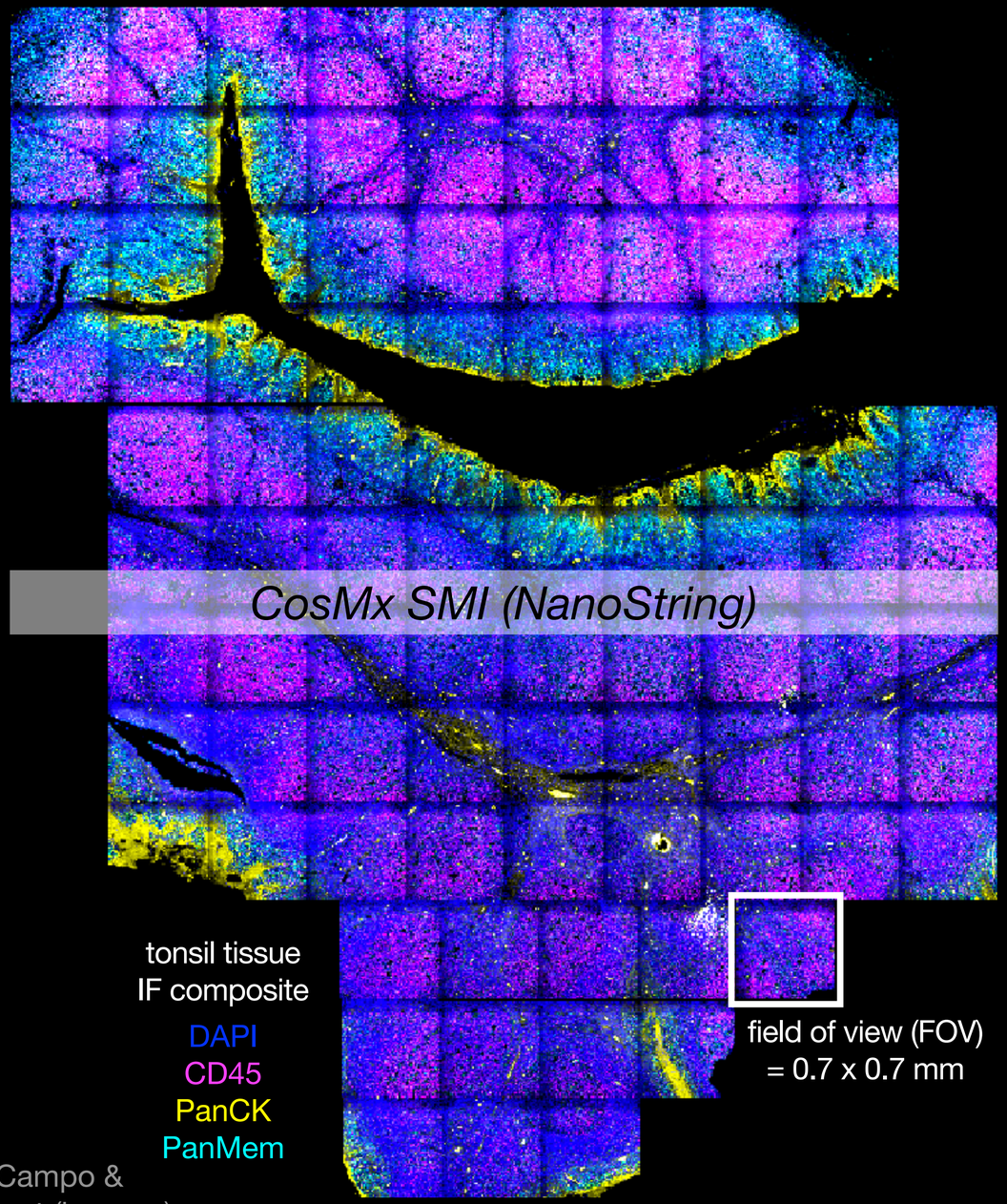
DAPI PanCK CD45 CD68



PC1 PC2 PC3

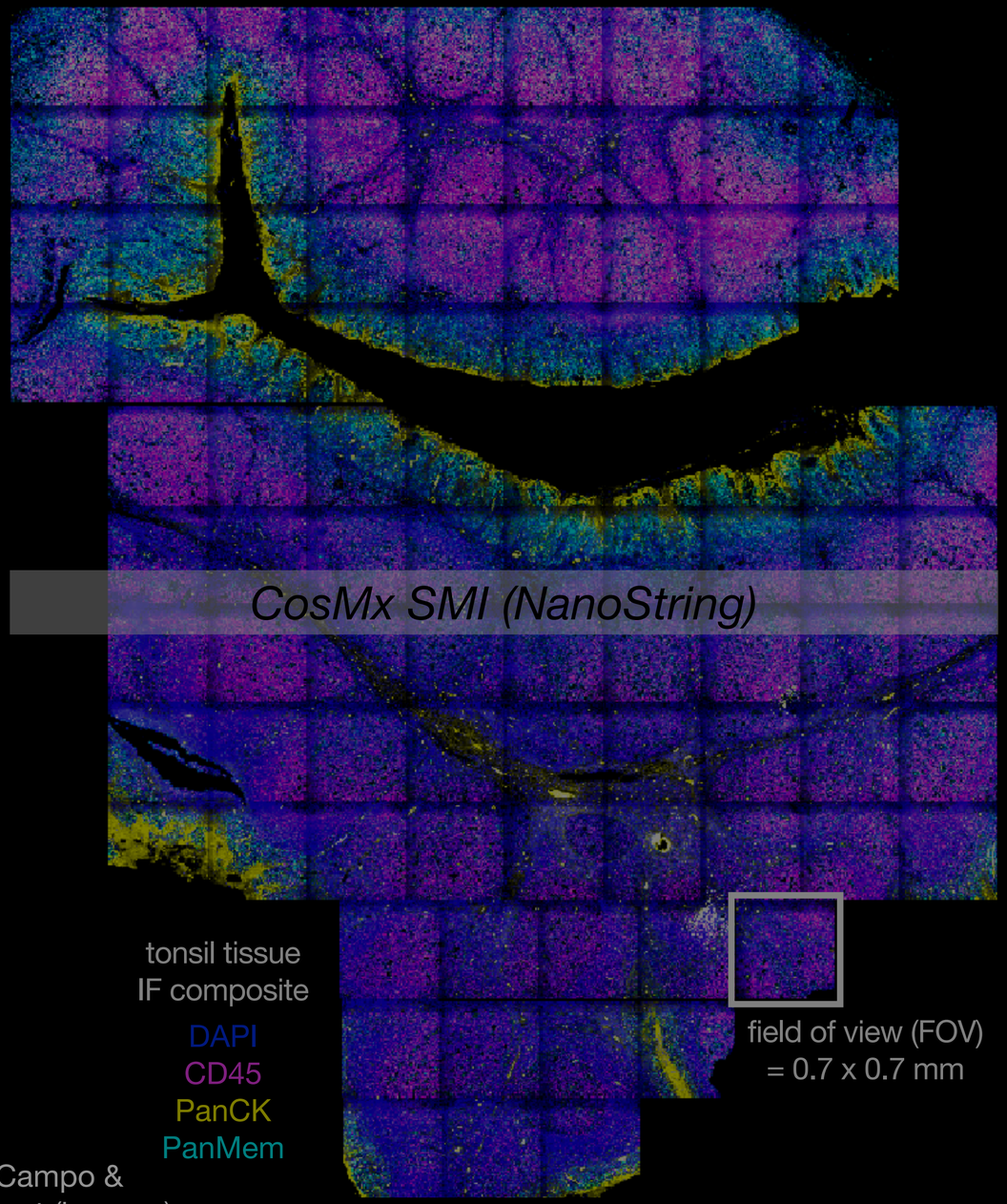
Crowell *et al.* Tejpar & Heyne (in prep.)

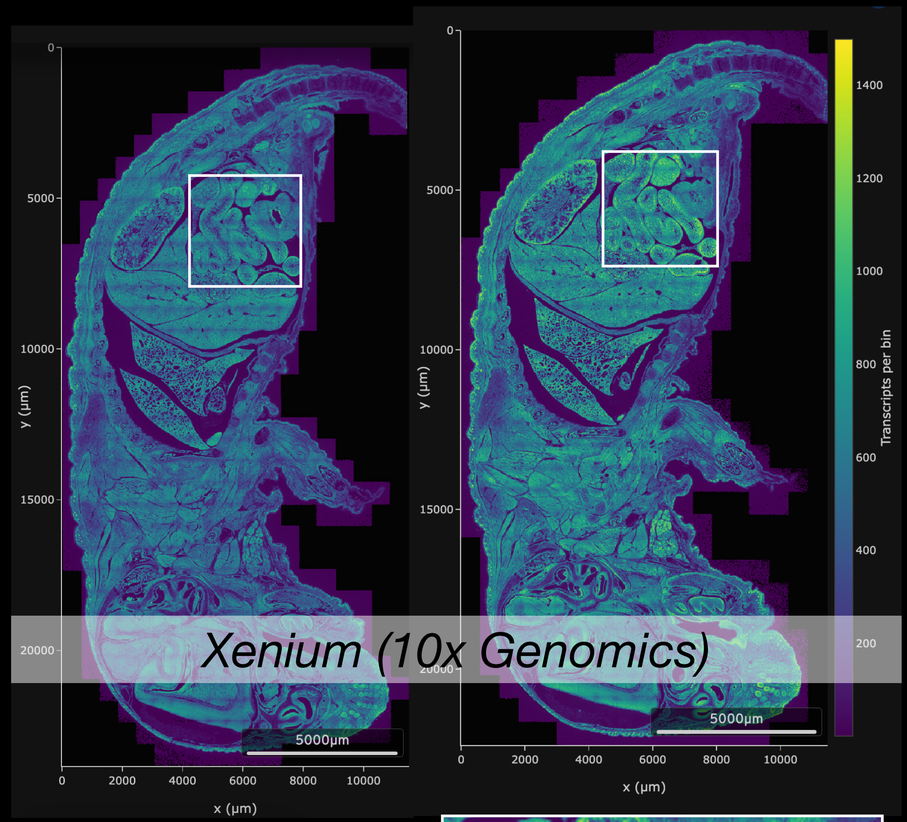
changes in optical performance lead to **lower optical resolution at FOV edges**

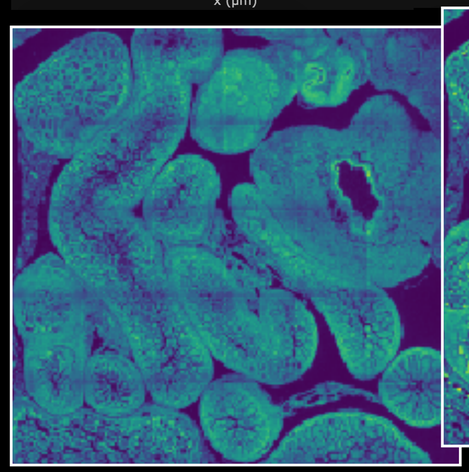


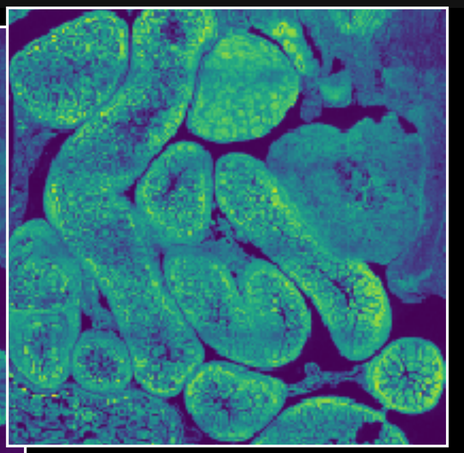
Crowell *et al.* Campo & Pascual-Reguant (in prep.)

changes in optical performance lead to **lower optical resolution at FOV edges**



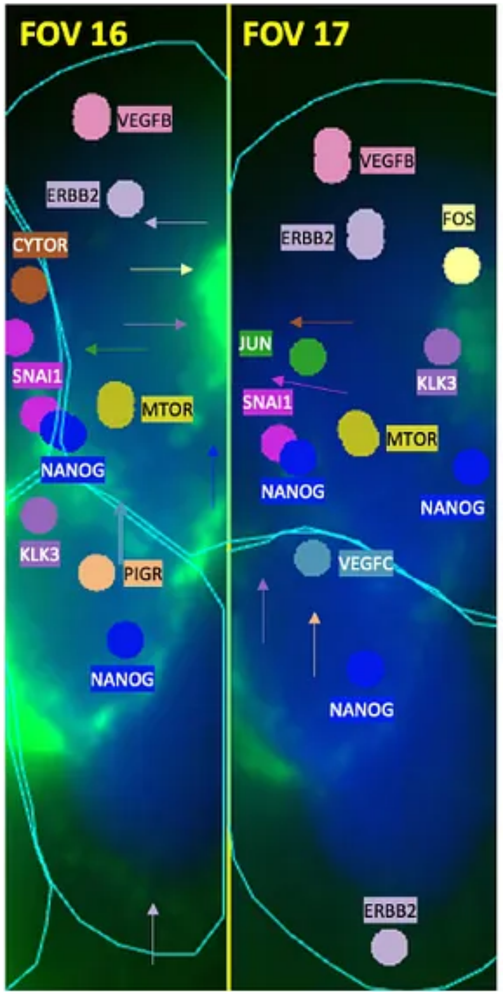


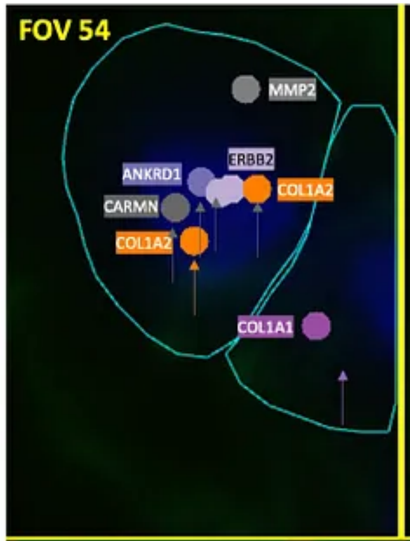


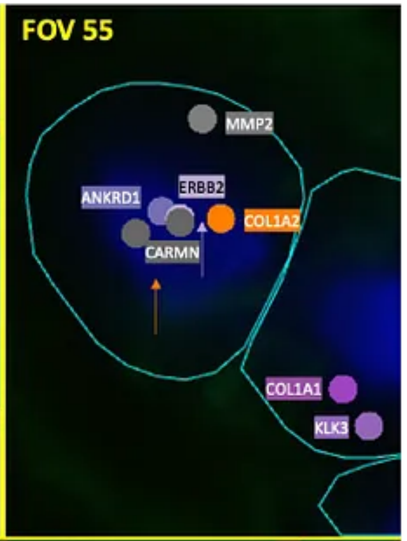


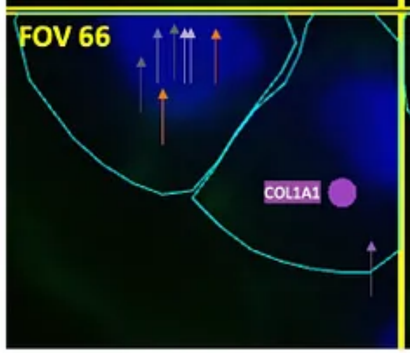
Crowell *et al.* Campo & Pascual-Reguant (in prep.)

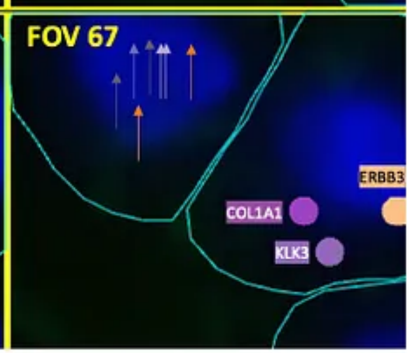
lack of stiching leads to cell **fragmentation, duplication & inconsistencies**

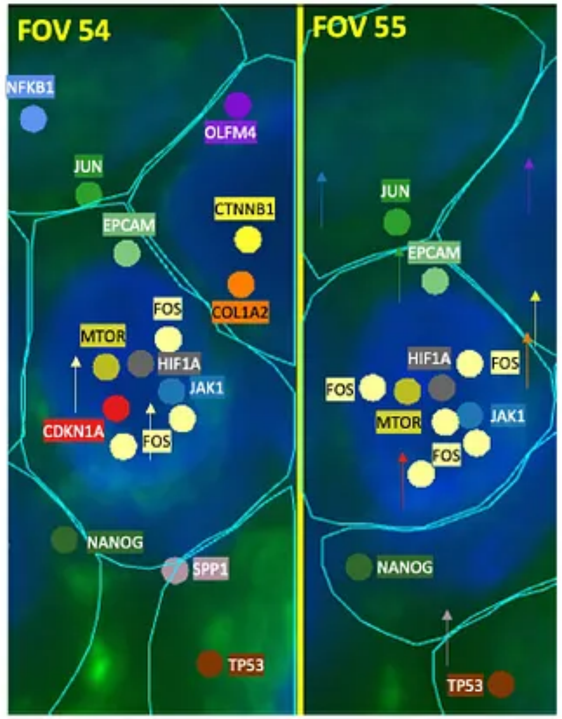


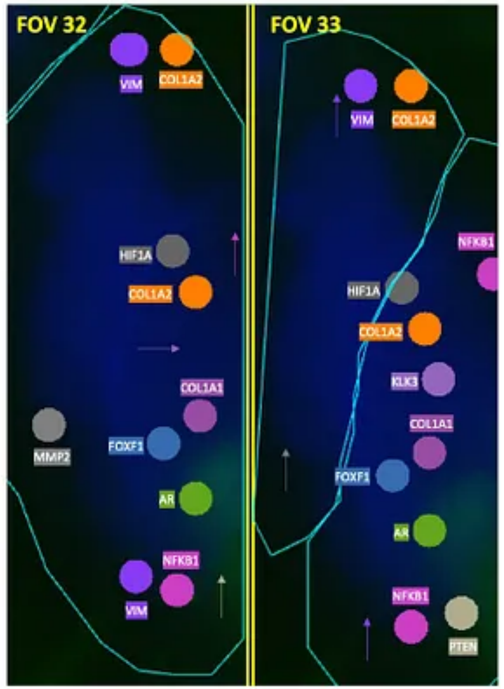








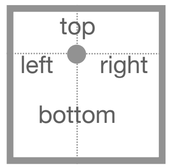




- arrows = missing transcripts
- partial cells are the same between FOVs, but come with unique IDs

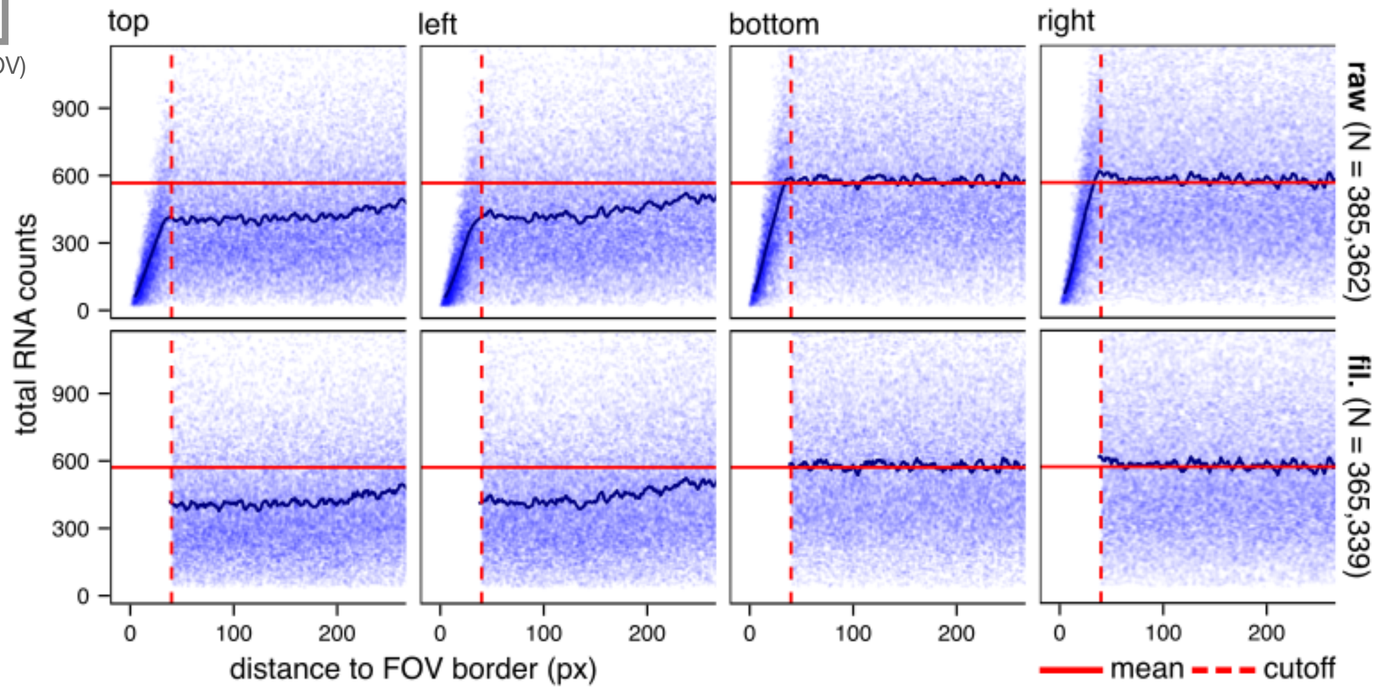
https://albertvilella.substack.com/p/comparison-between-10x-genomics-xenium

border cells have fewer counts & can highlight other artefacts



field of view (FOV)

- compute each cell's distance to each FOV border
- plot counts vs. distance, stratified by direction



points = cells (across all FOVs), lines = running median

Crowell *et al.* Campo & Pascual-Reguant (in prep.)

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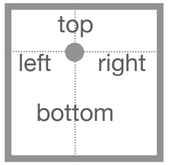

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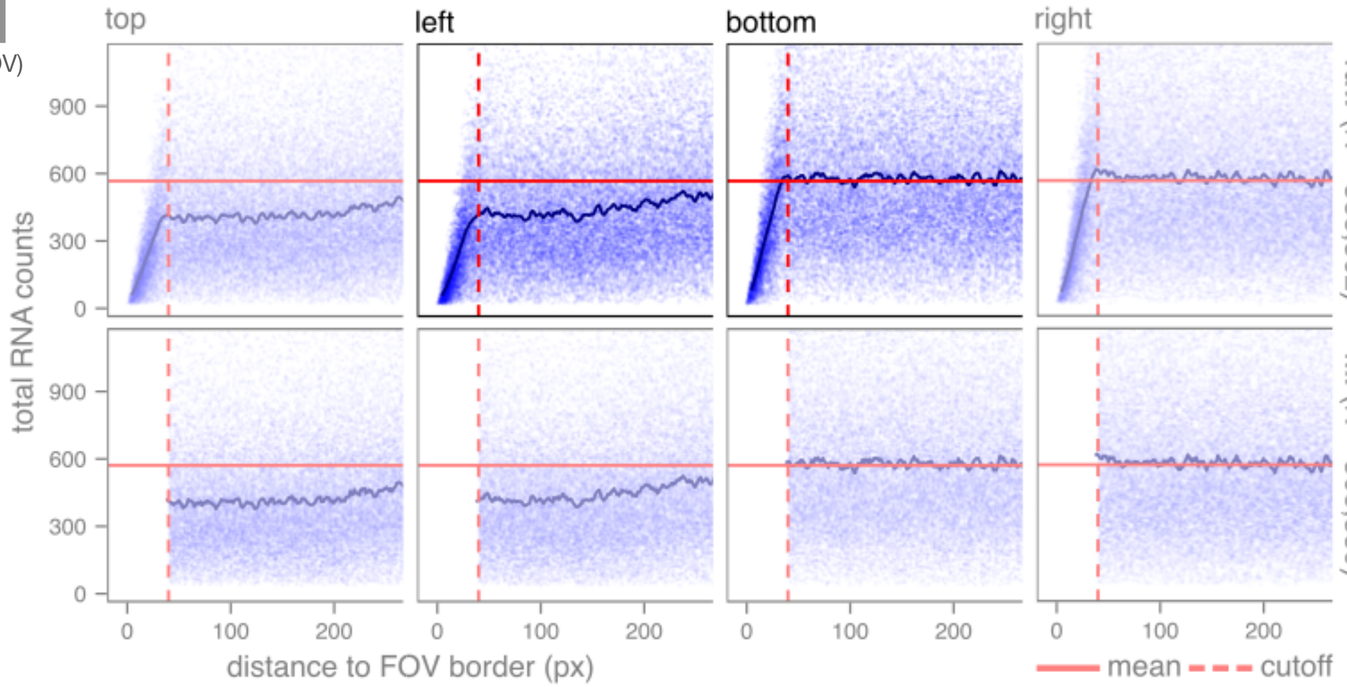

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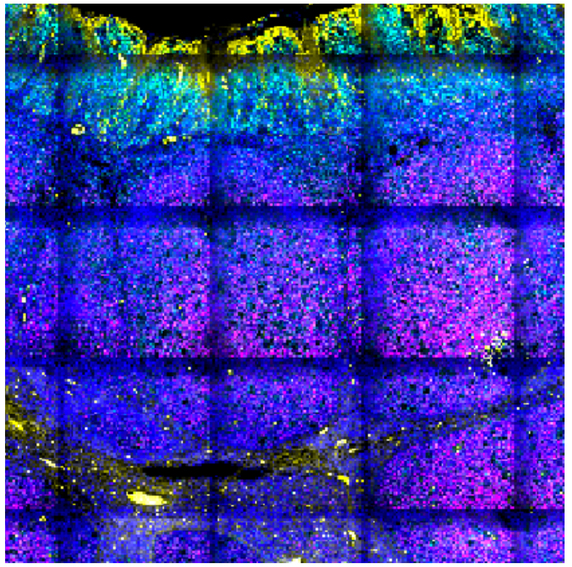
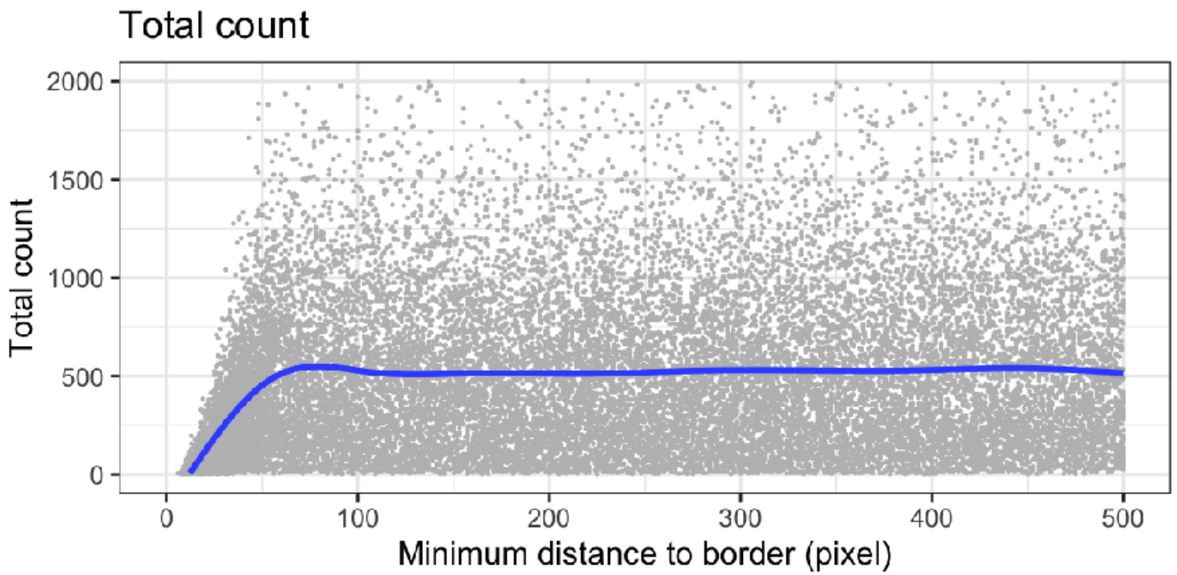
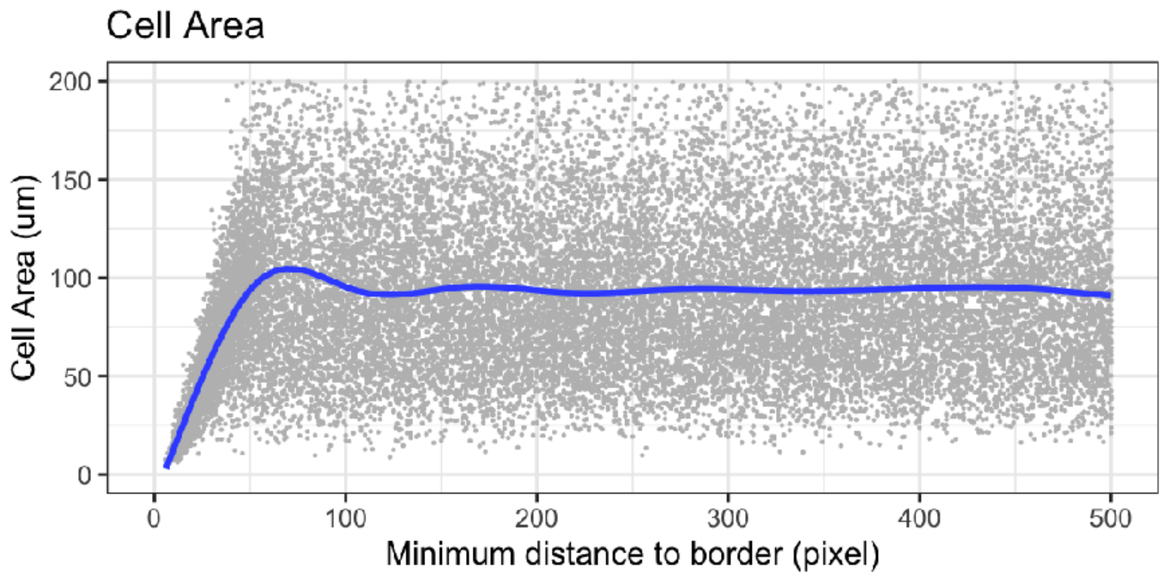


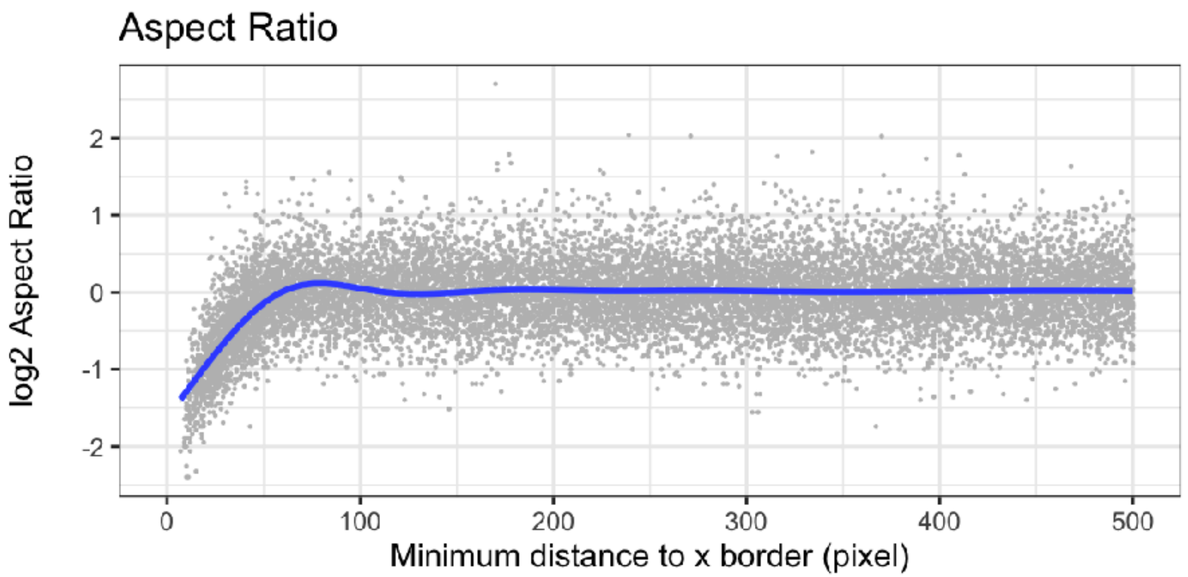
top-left corners exhibit dimmer IF signals

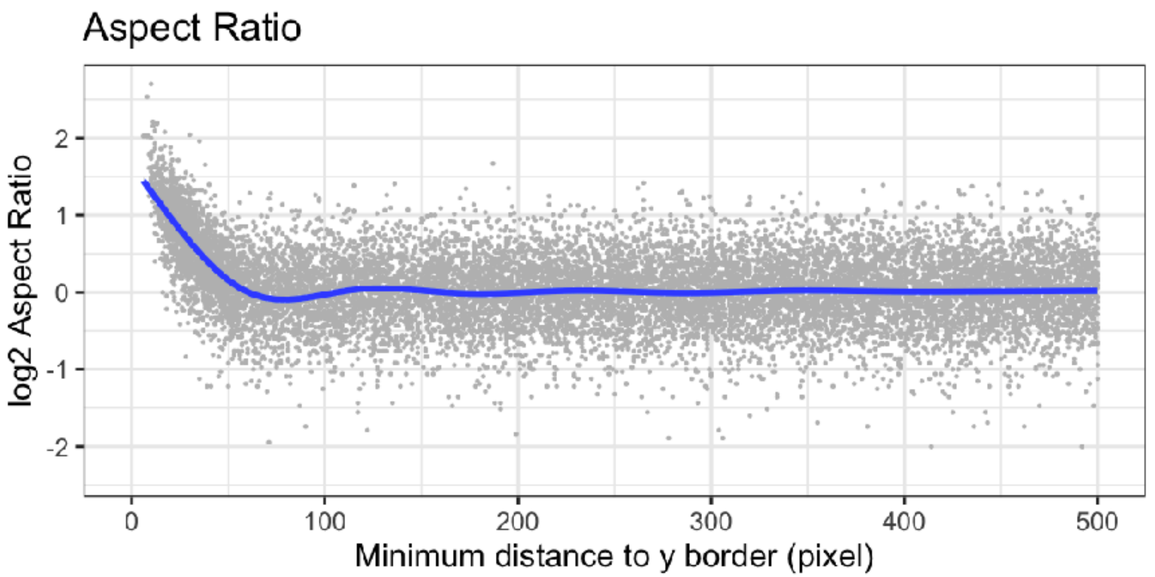
Crowell *et al.* Campo & Pascual-Reguant (in prep.)

besides fewer counts, **border cells are smaller & slimmer**





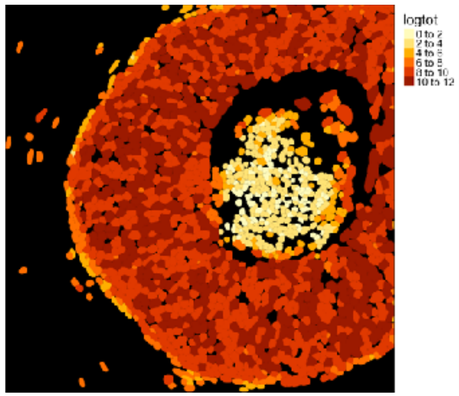




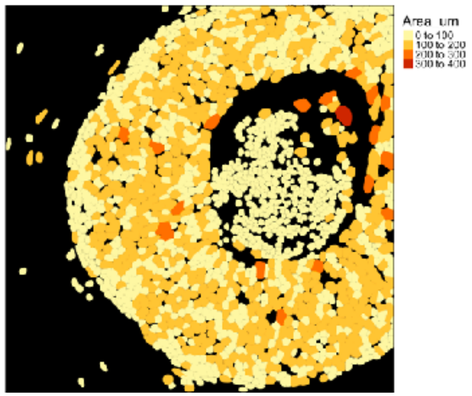
plots courtesy of Davide Risso

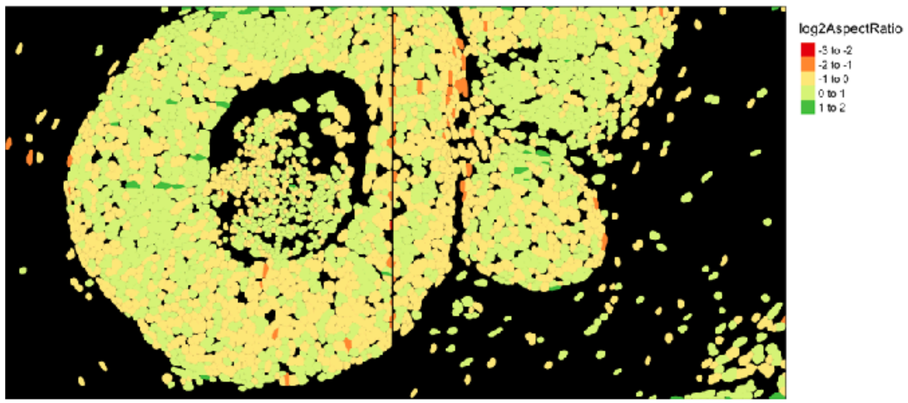
SpaceTrooper proposes a *flag score* combining several metrics

counts



area



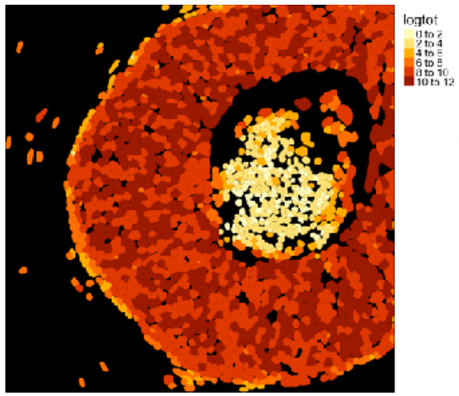


aspect ratio

https://github.com/drighelli/SpaceTrooper

SpaceTrooper proposes a *flag score* combining several metrics

counts



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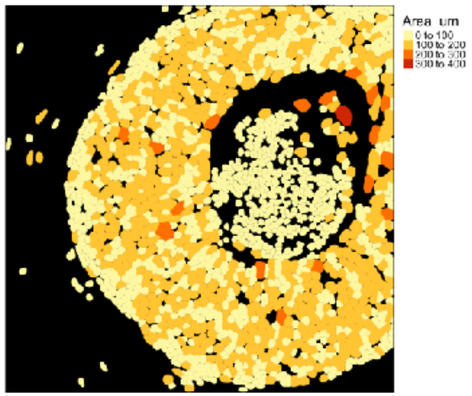
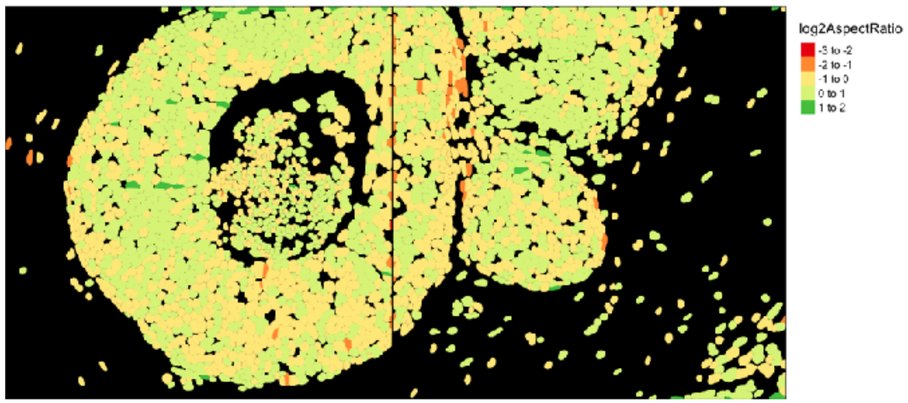
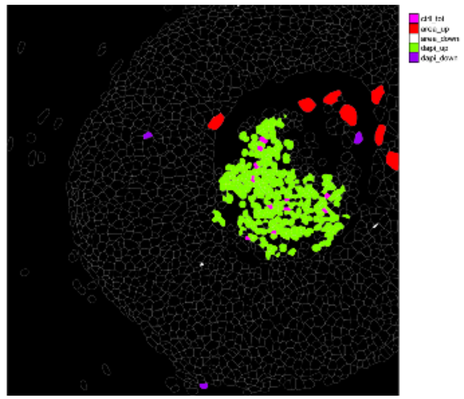




aspect ratio

outliers



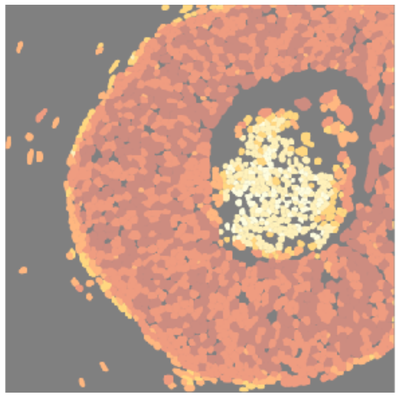
(e.g., thresholding on MADs of univariate distributions)

https://github.com/drighelli/SpaceTrooper

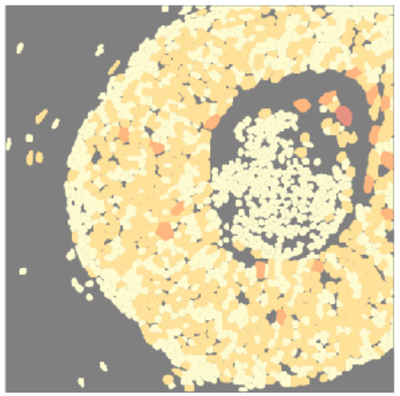
SpaceTrooper proposes a *flag score* combining several metrics

$$\text{logit}(F) = \beta_0 + \beta_1 \log \frac{\text{count}}{\text{area}} + \beta_2 \left| \log(\text{aspect ratio}) \right| \cdot I_{\{d<\text{threshold}\}}$$

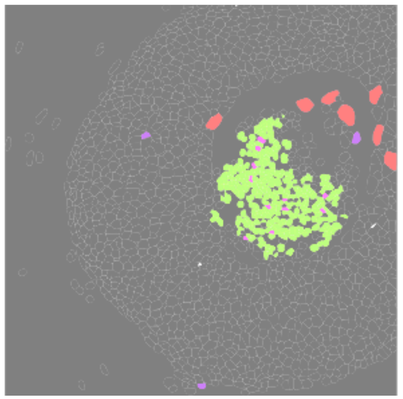
counts



area



outliers



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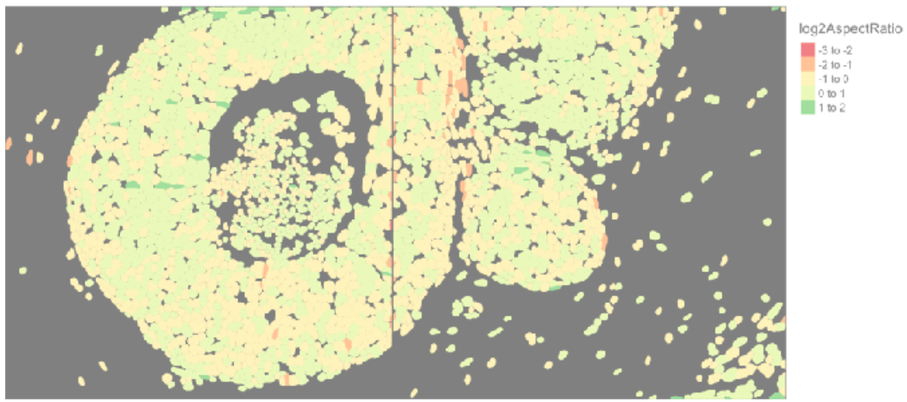

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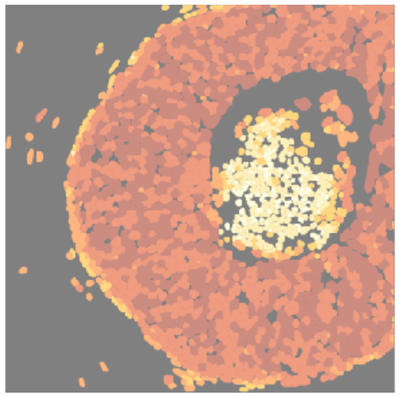
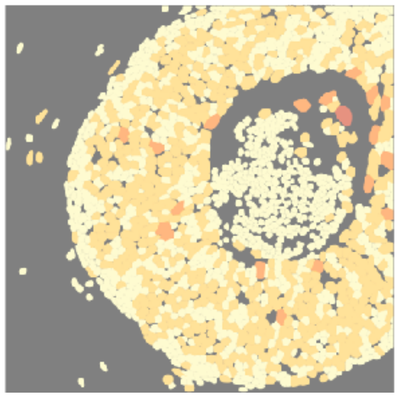
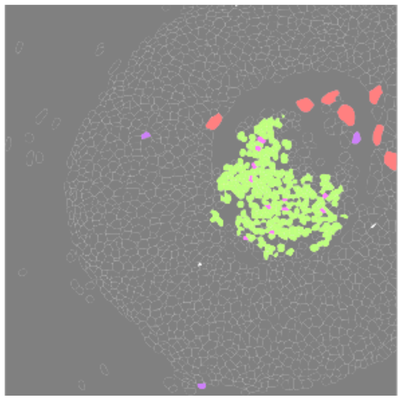
counts

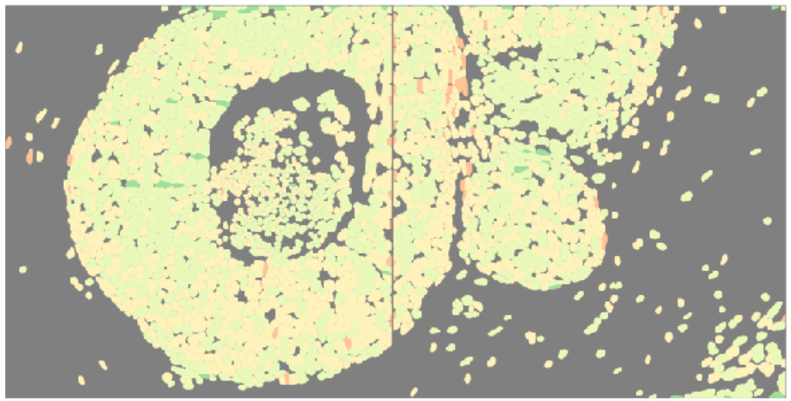


area

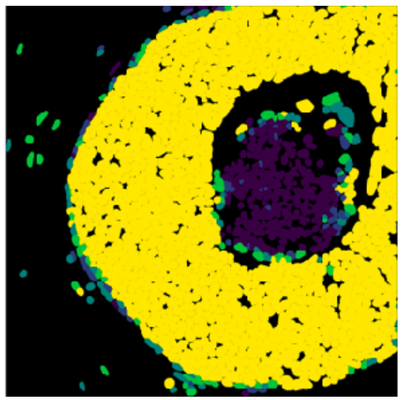


outliers





aspect ratio



flag score

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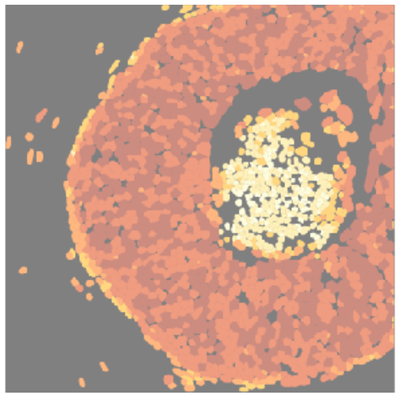
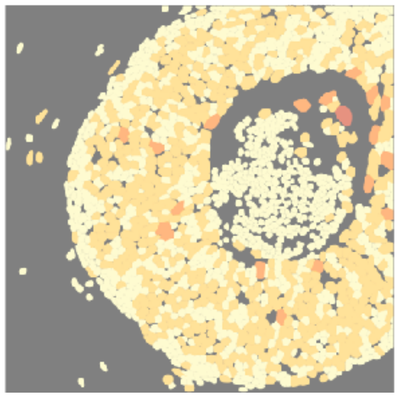
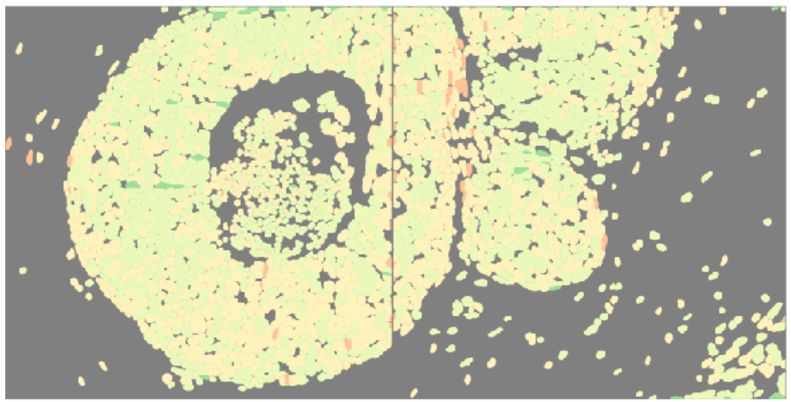
counts



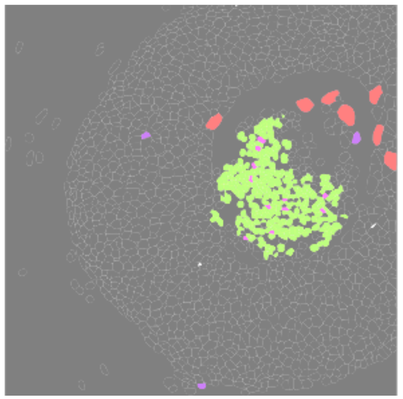
area

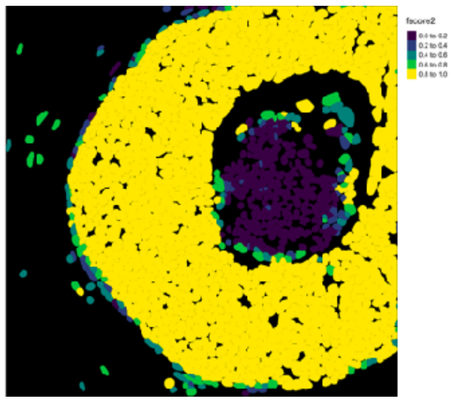




aspect ratio

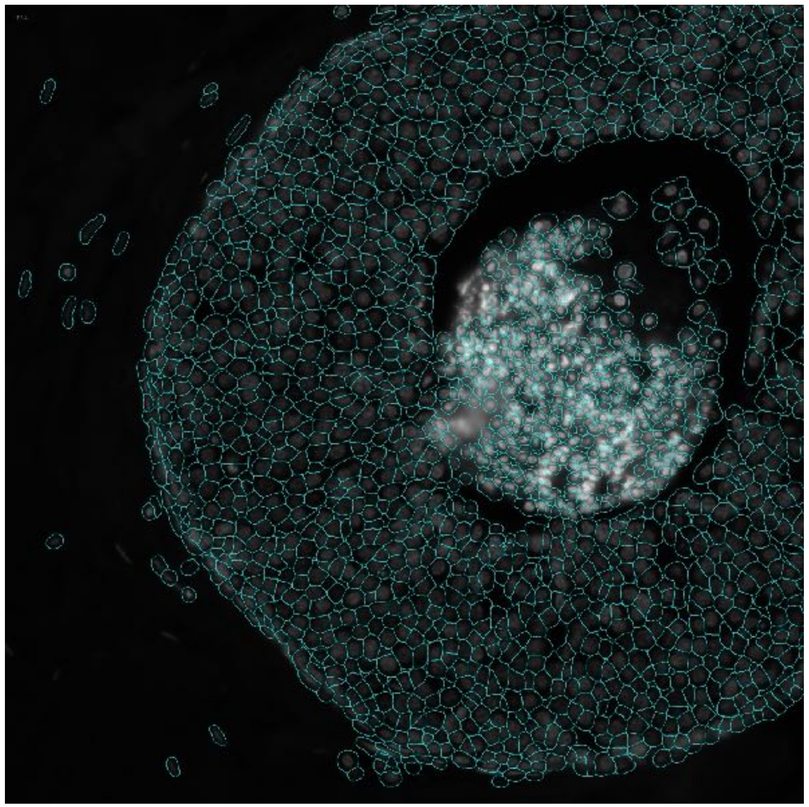
outliers





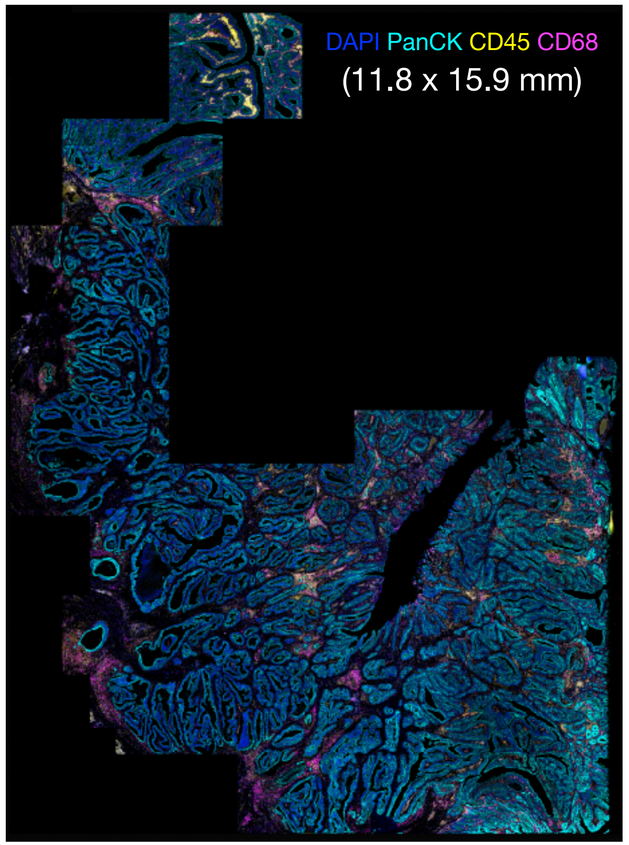
flag score

DAPI + segmentation



https://github.com/drighelli/SpaceTrooper

debris is usually segmented, yet hard to distinguish



Crowell *et al.* Tejpar & Heyne (in prep.)

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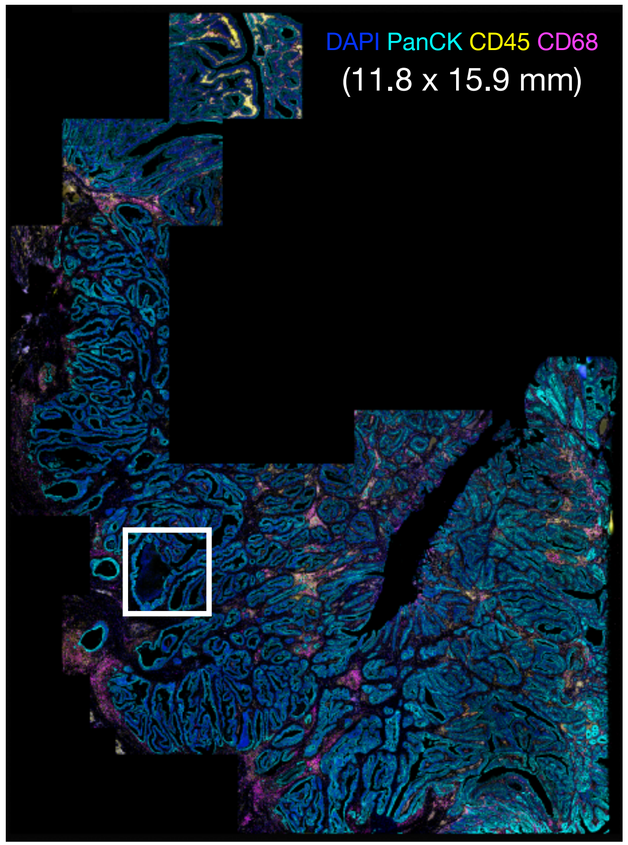

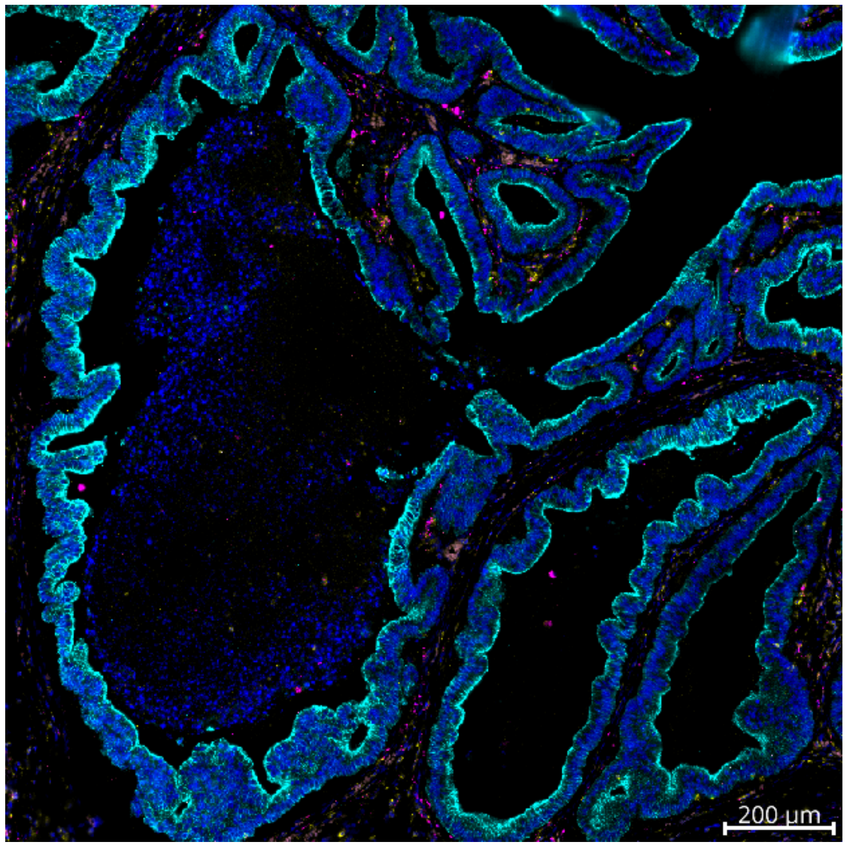

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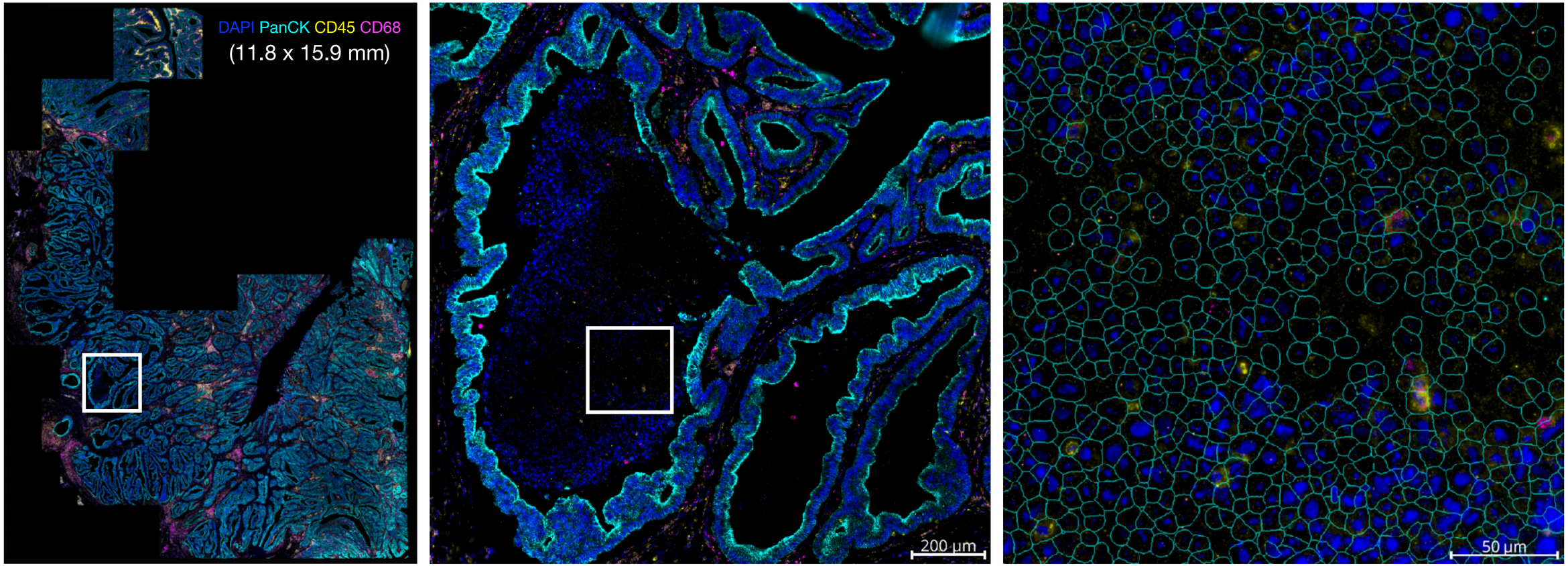

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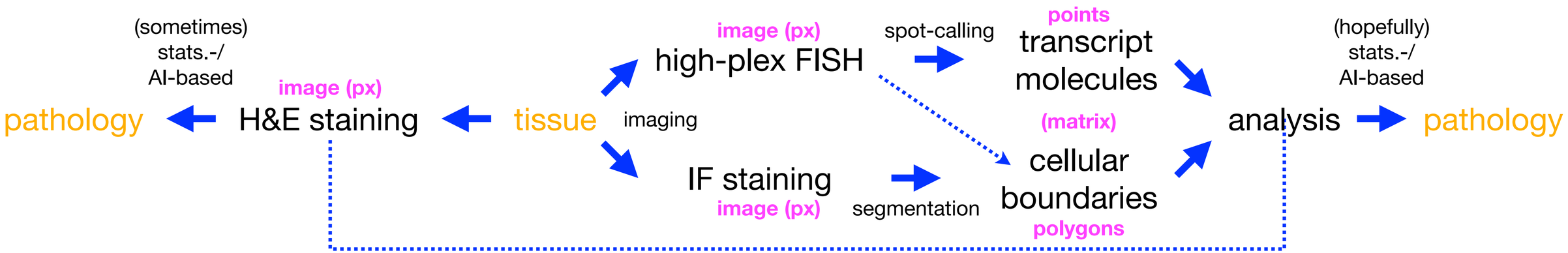
library size normalization on non-representative panels introduces biases



Atta *et al.* (2024), *Genome Biology* 25:153

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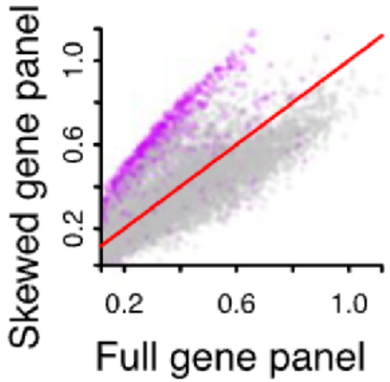

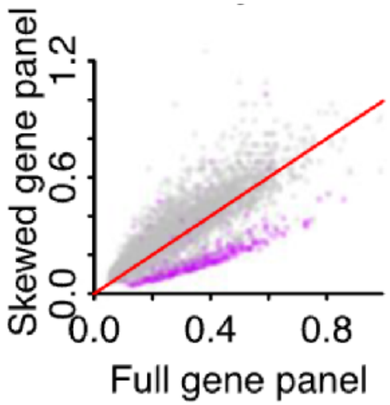

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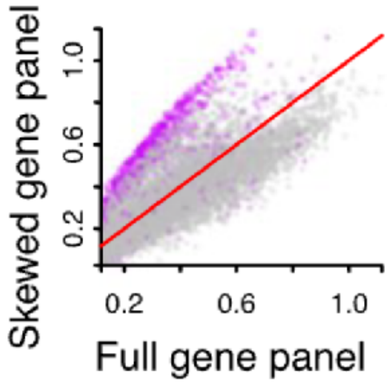
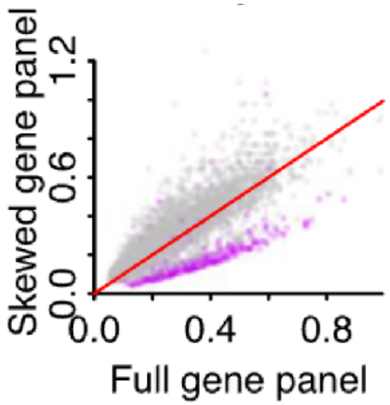
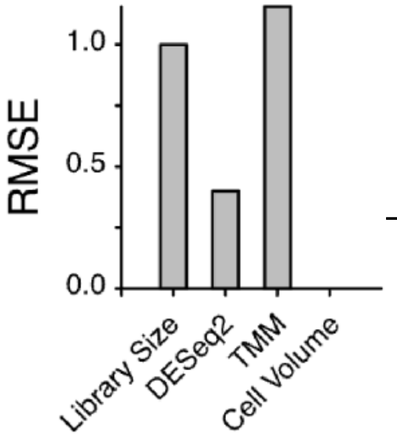
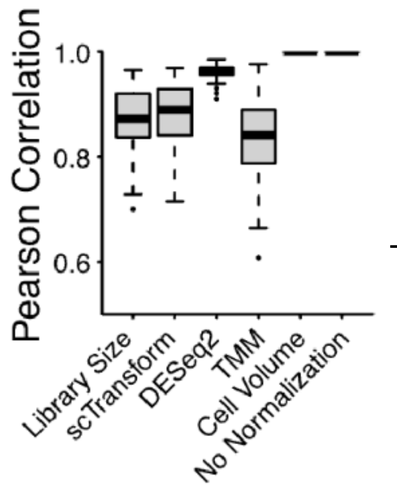




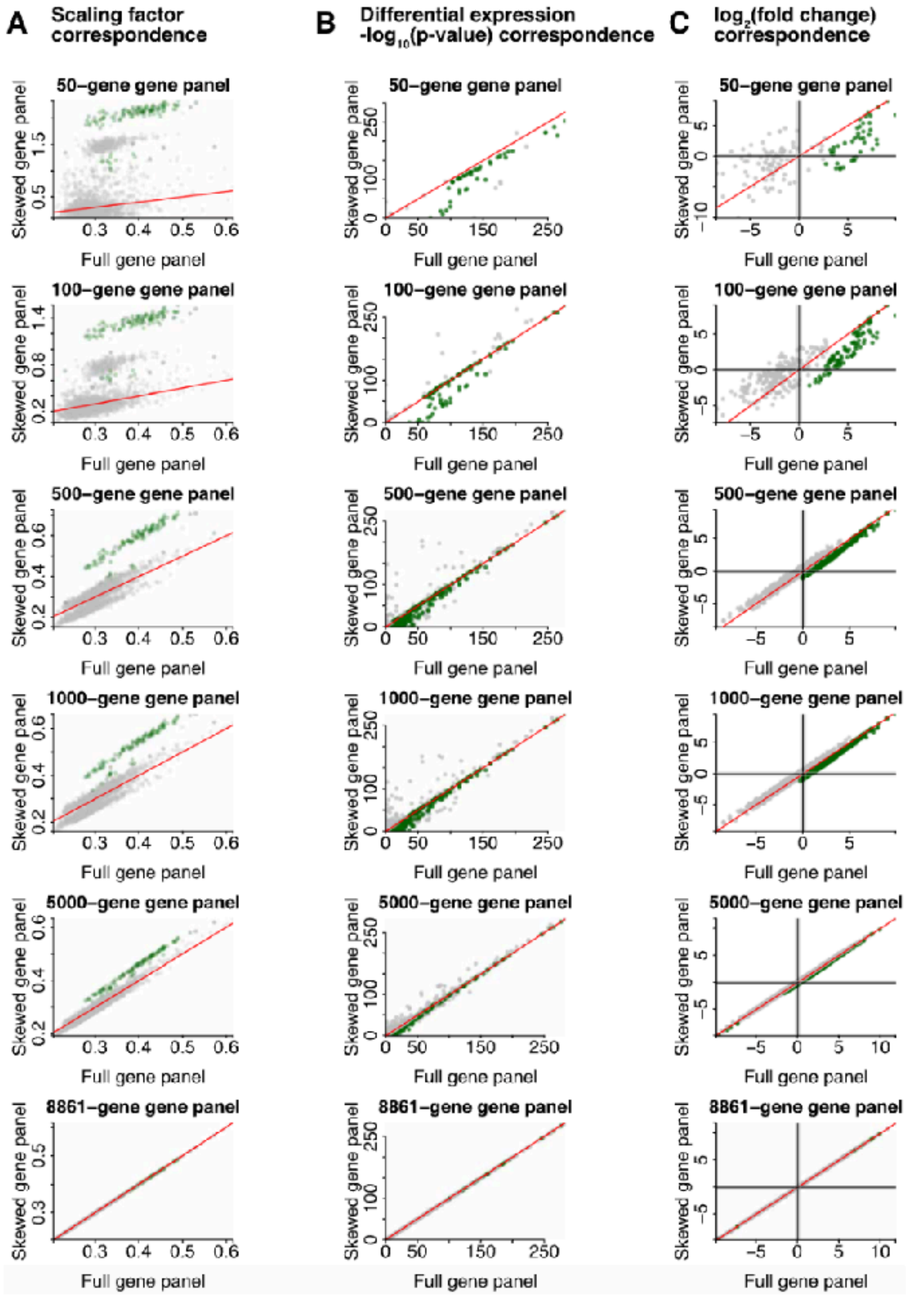




- systematic biases affect analyses to evaluate **differential gene expression & spatially variable genes**

Atta *et al.* (2024), *Genome Biology* 25:153

larger/more representative panels help **mitigate region-specific effects**



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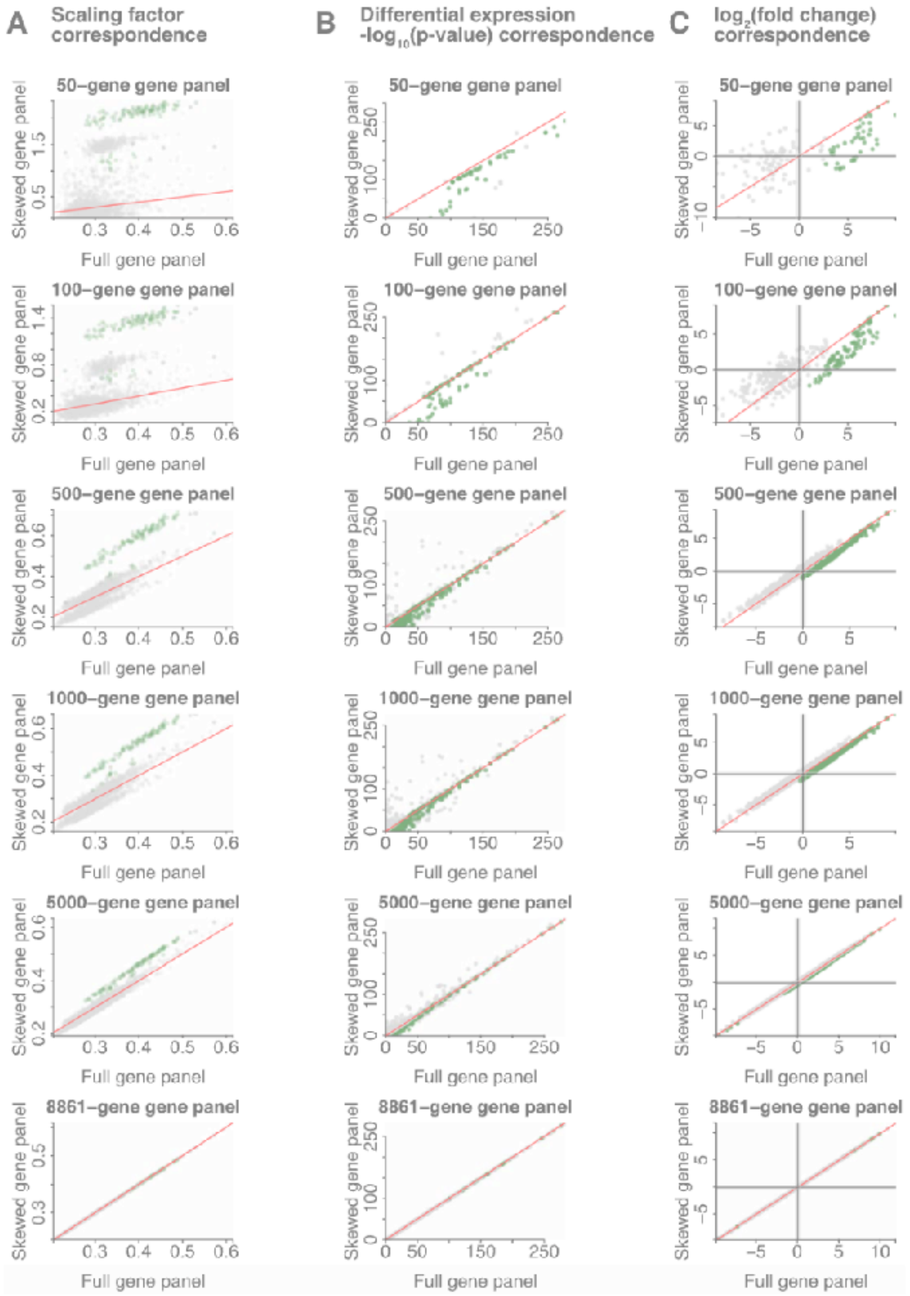

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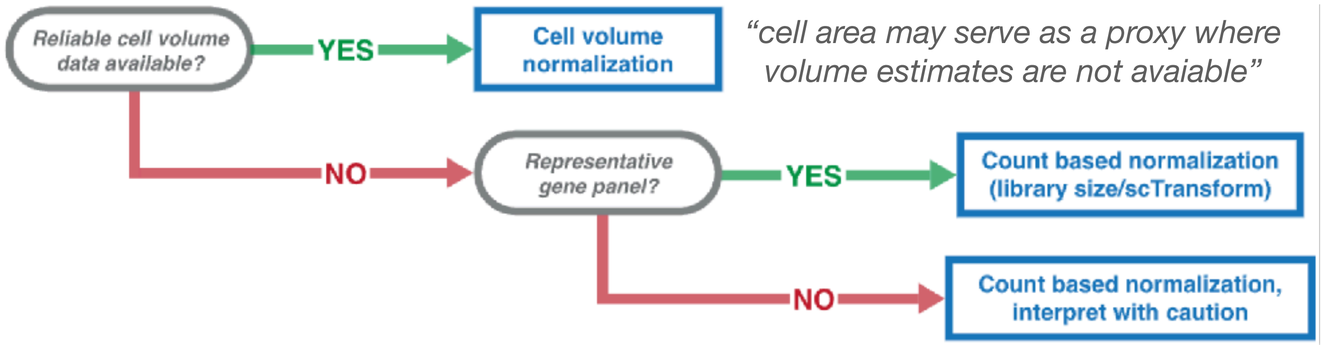
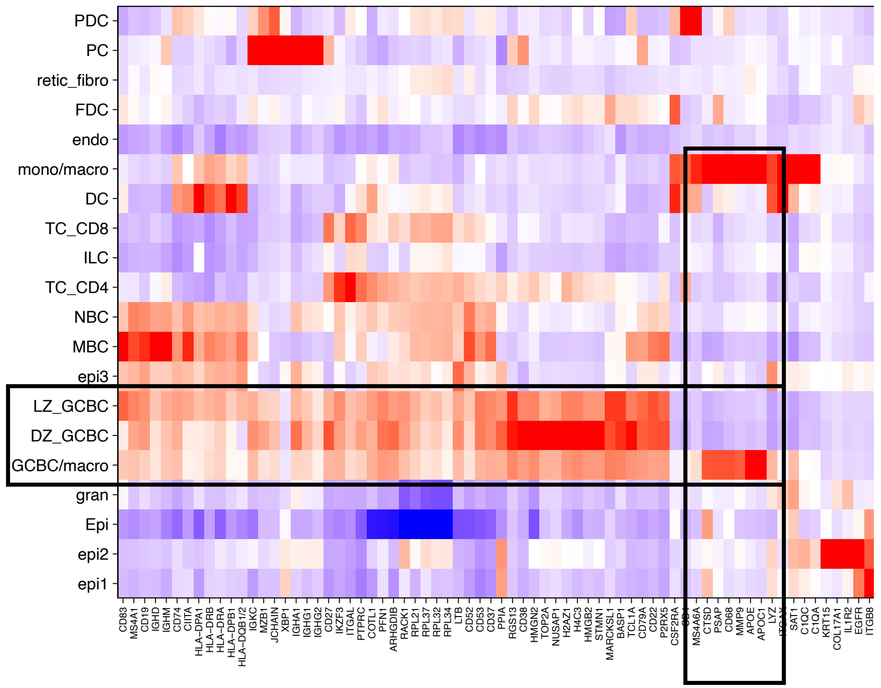


Atta *et al.* (2024), *Genome Biology* 25:153

CD68+ B cells — what's going on here?



Crowell *et al.* Campo & Pascual-Reguant (in prep.)

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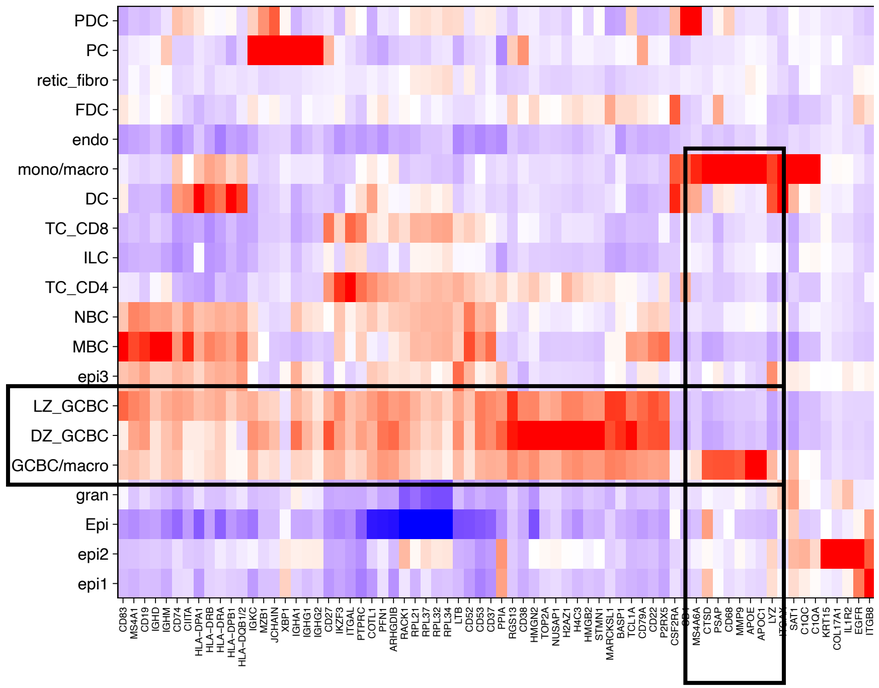
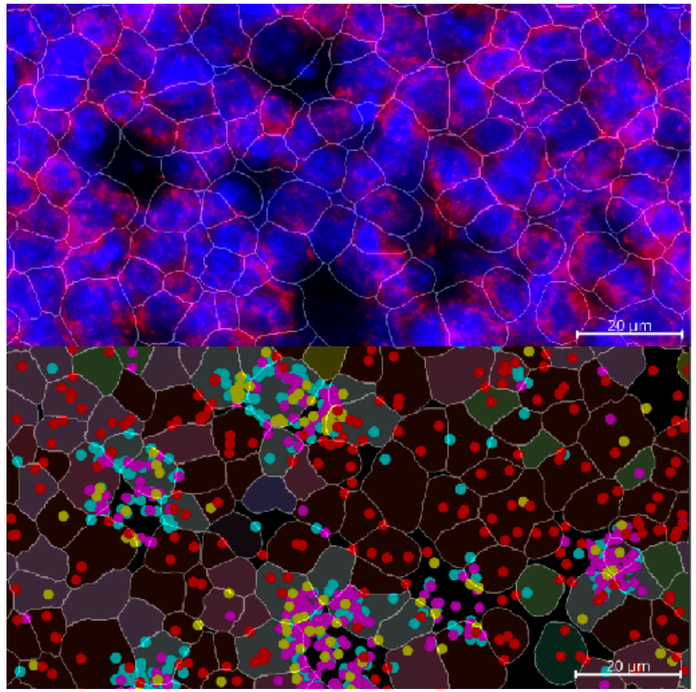


DAPI CD45



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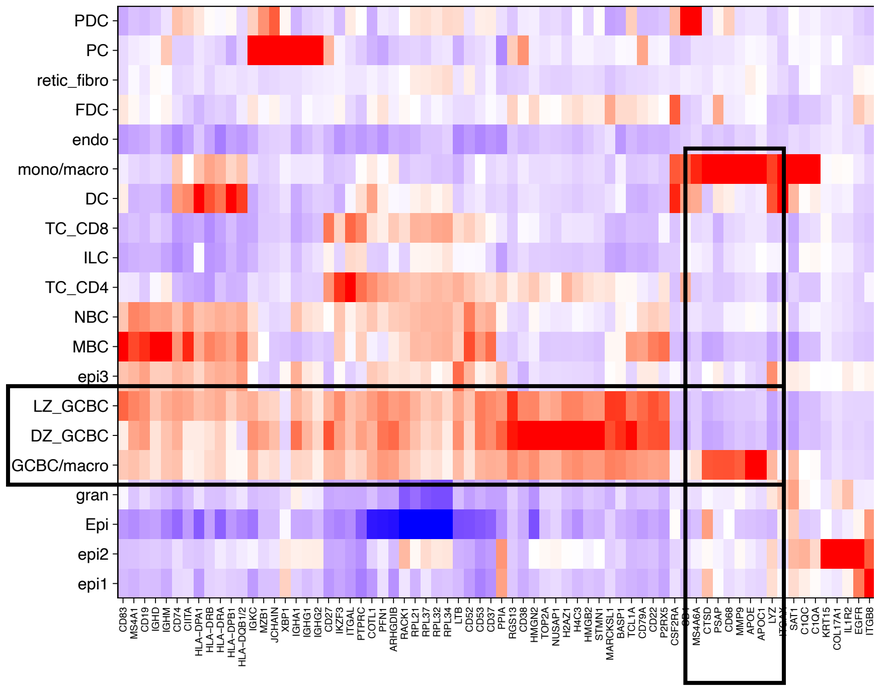

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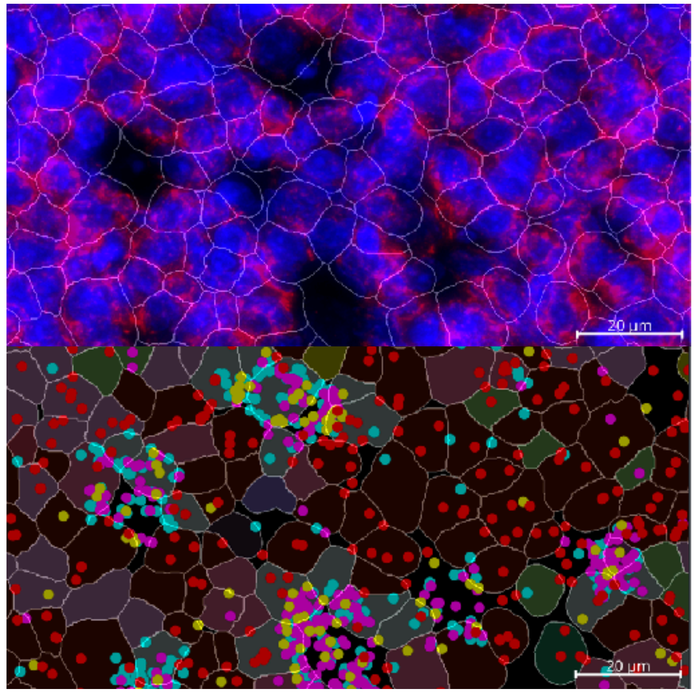

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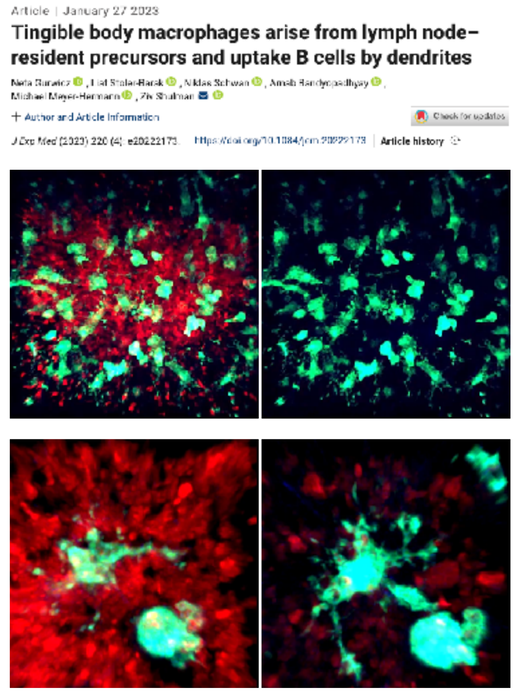
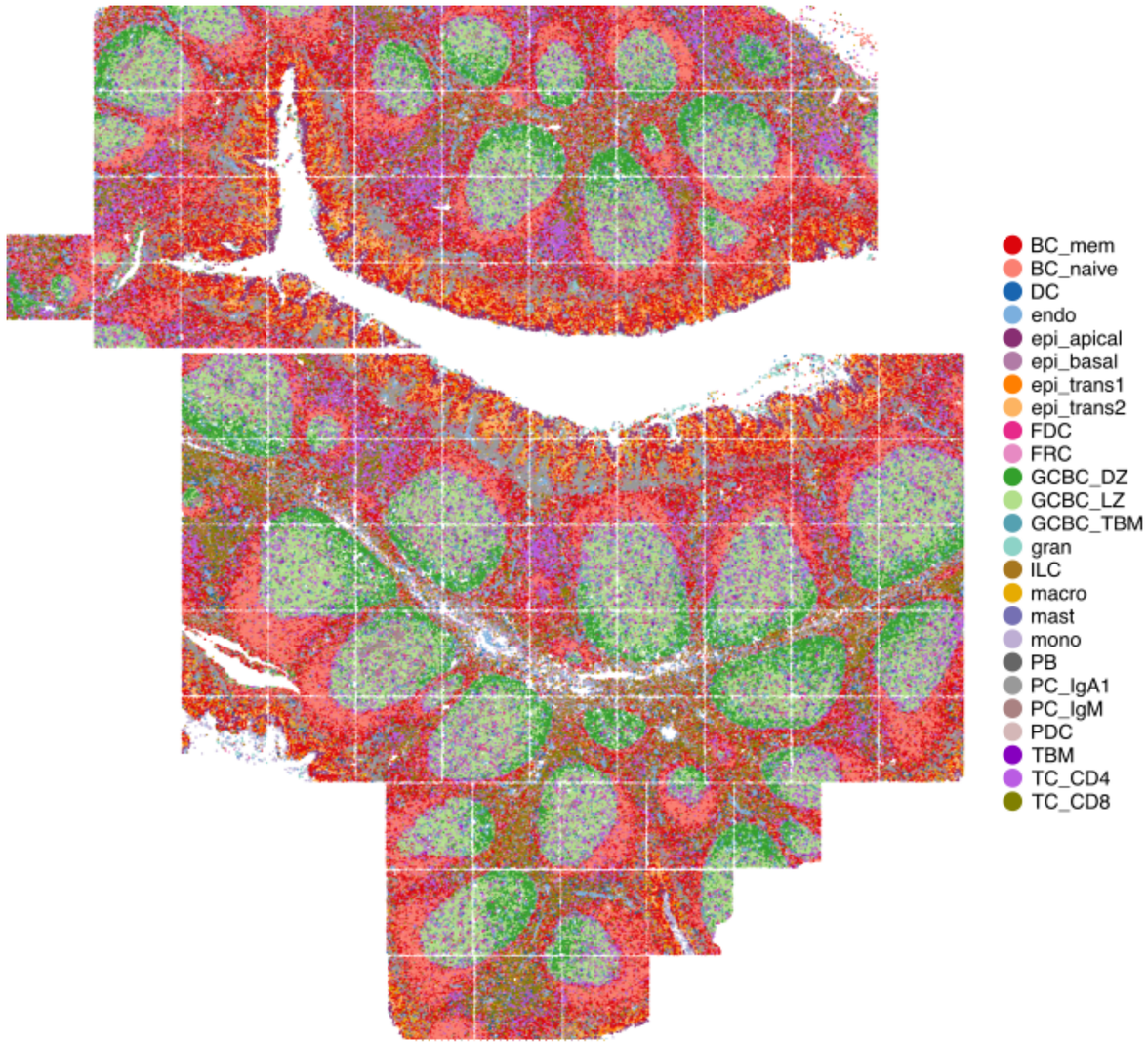


B cells macrophages

Crowell *et al.* Campo & Pascual-Reguant (in prep.)

spatial bleeding manifests in RNA counts, hence PCs, and UMAPs



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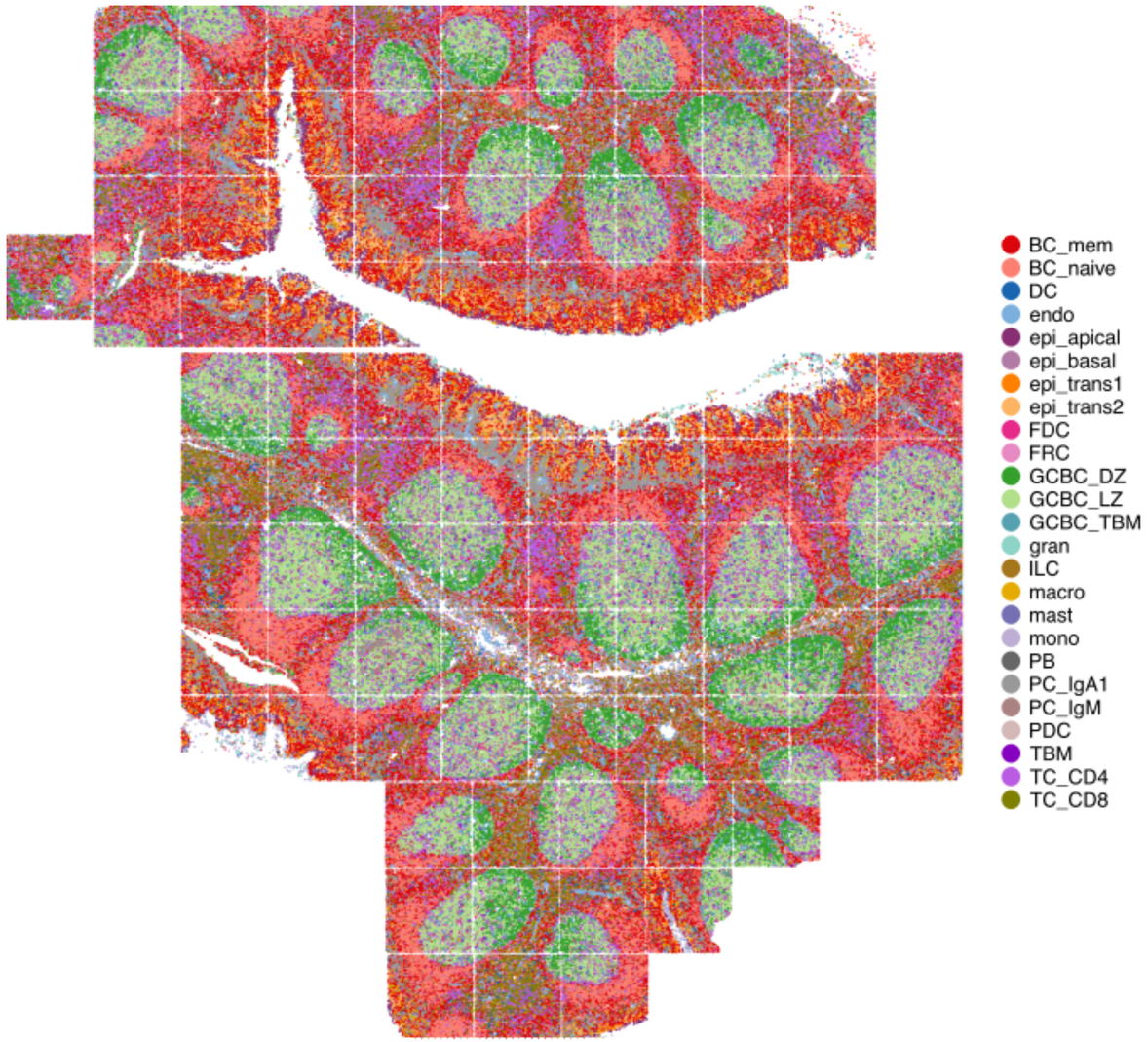

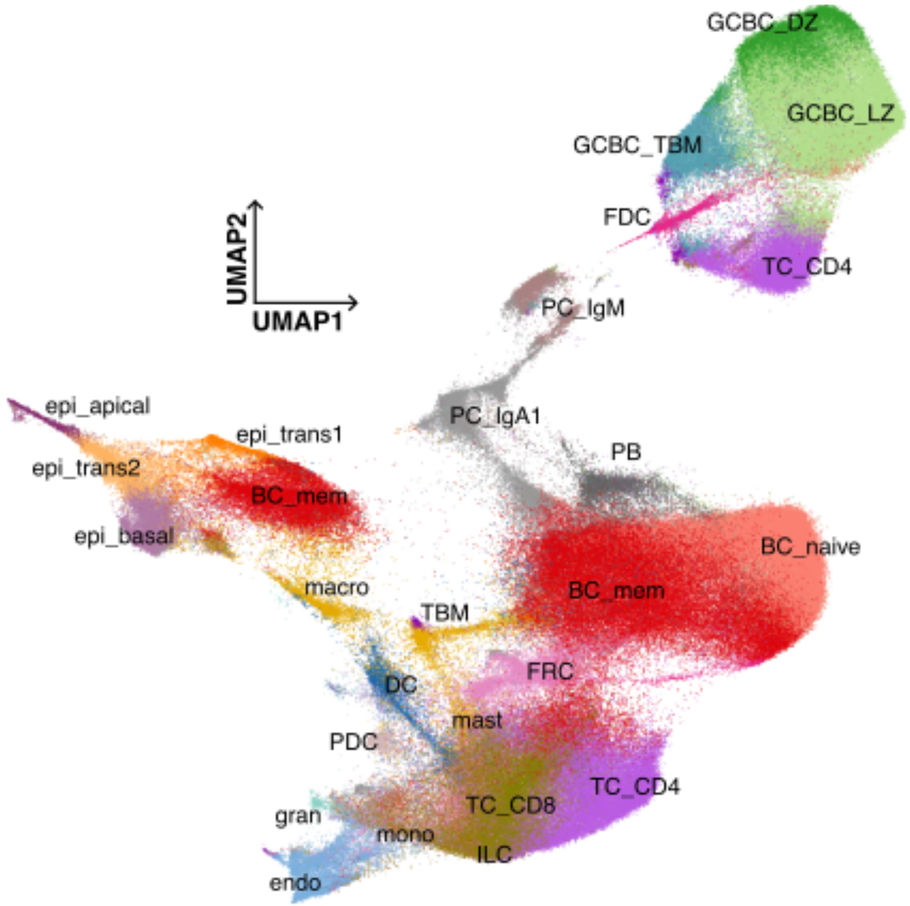

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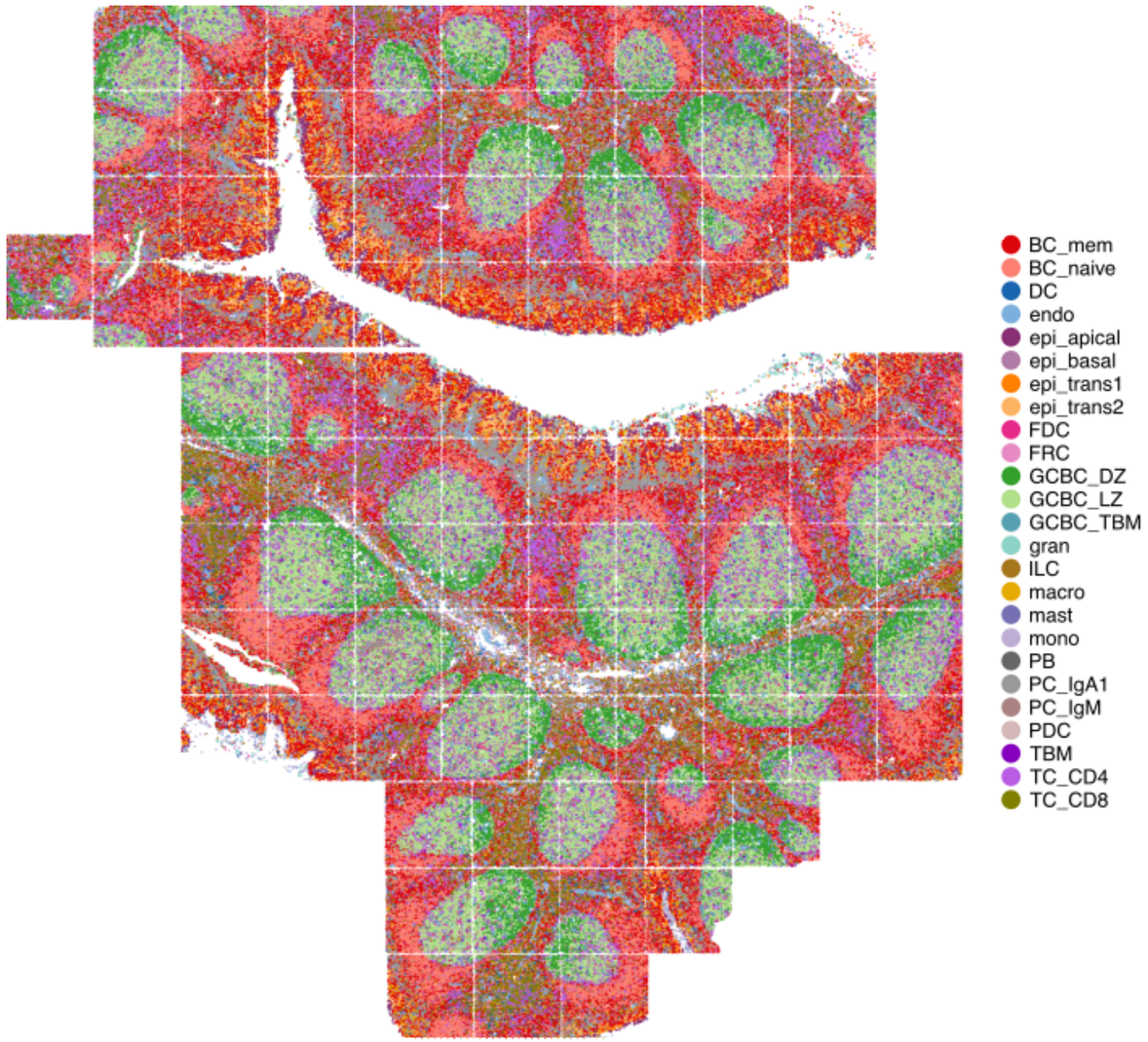

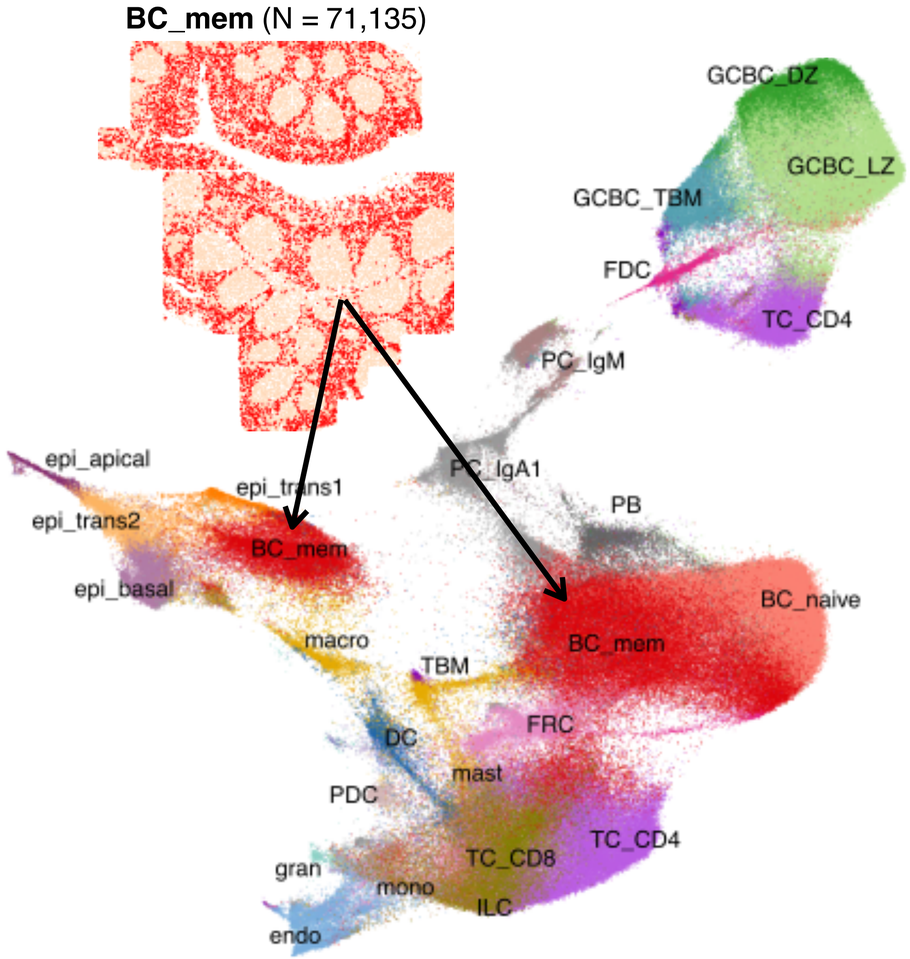

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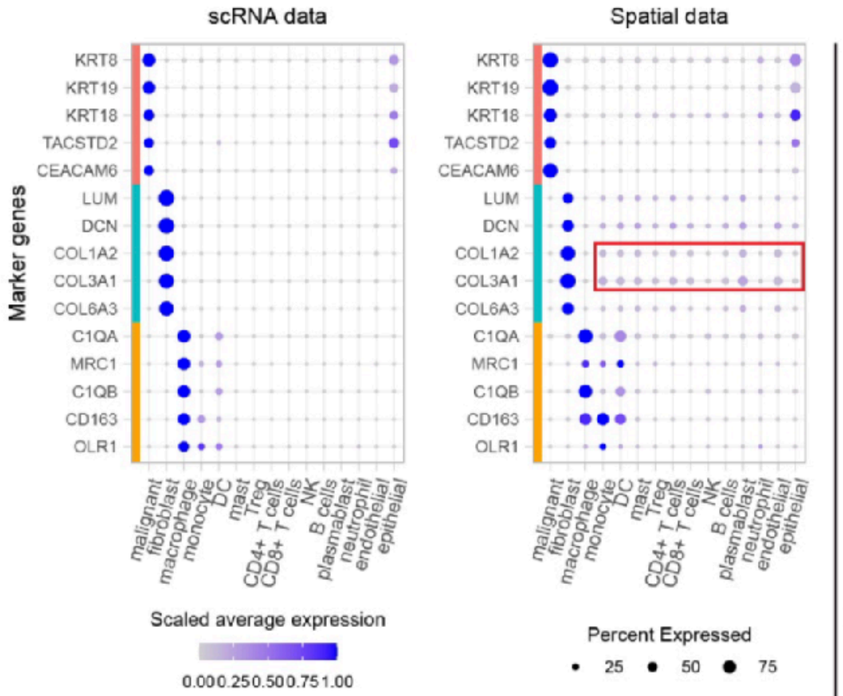
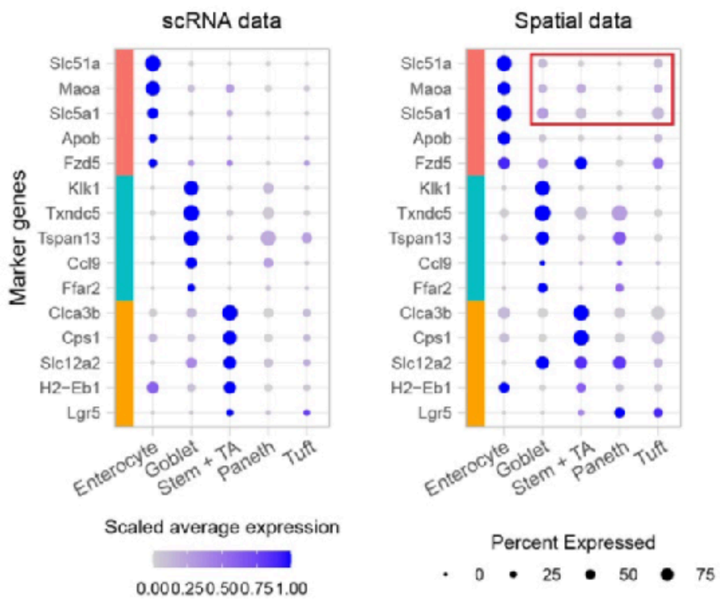
bleeding occurs in 3D — around, above & below cells

malignant fibroblast macrophage



enterocyte goblet stem+TA



Mitchel *et al.* (2024), *BioRxiv* 2025.01.02.631135

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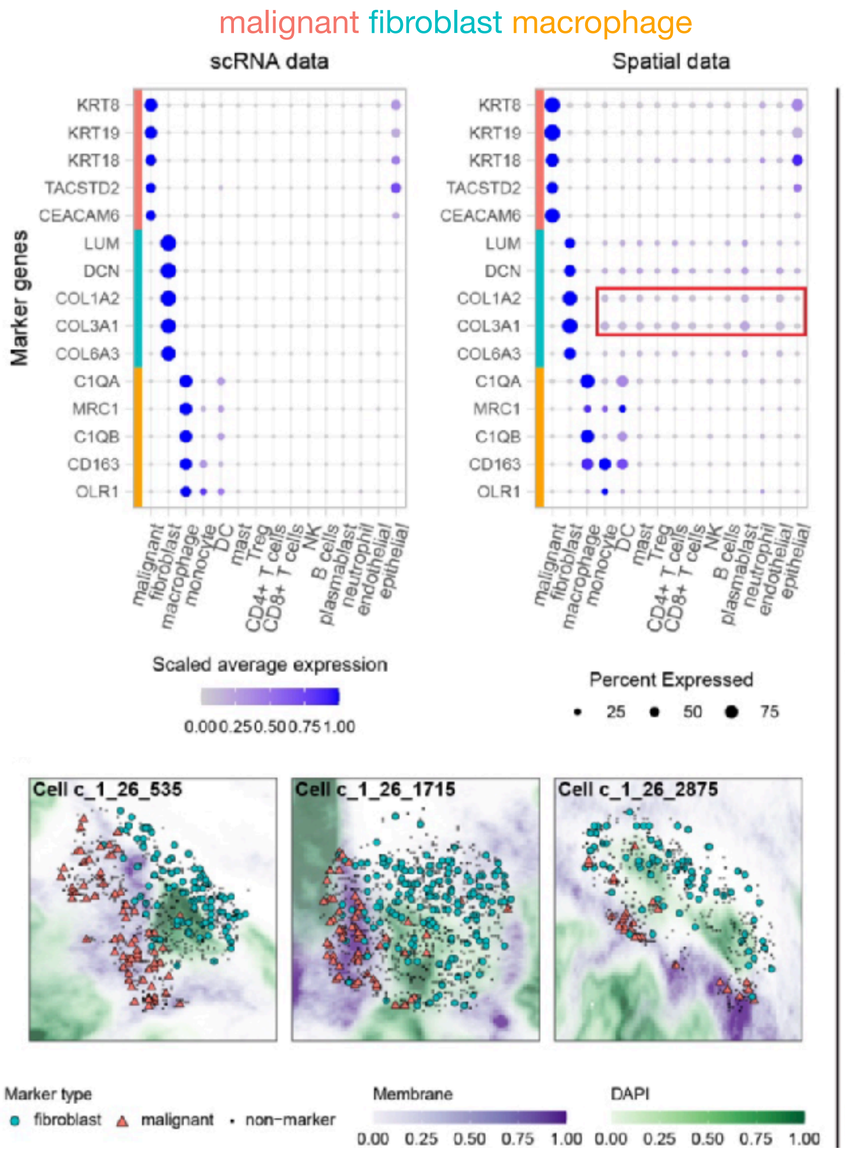
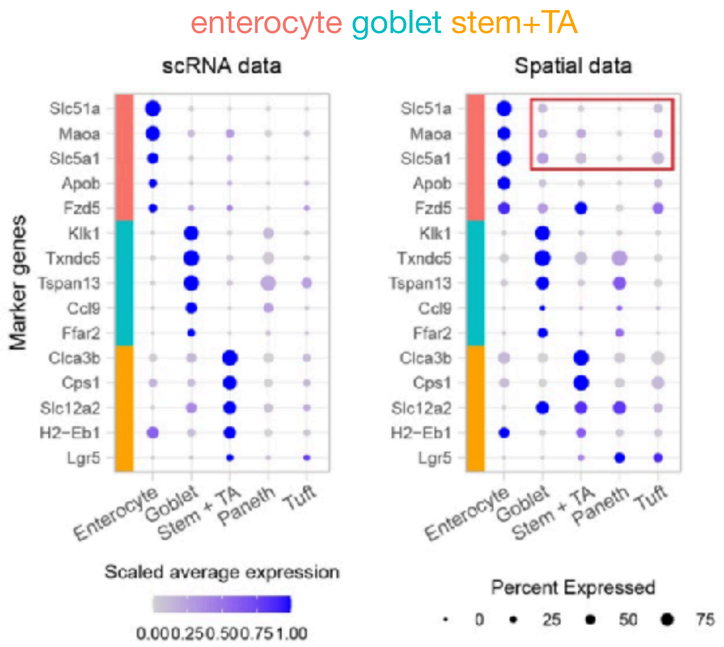
admixtures occur at **cellular periphery**





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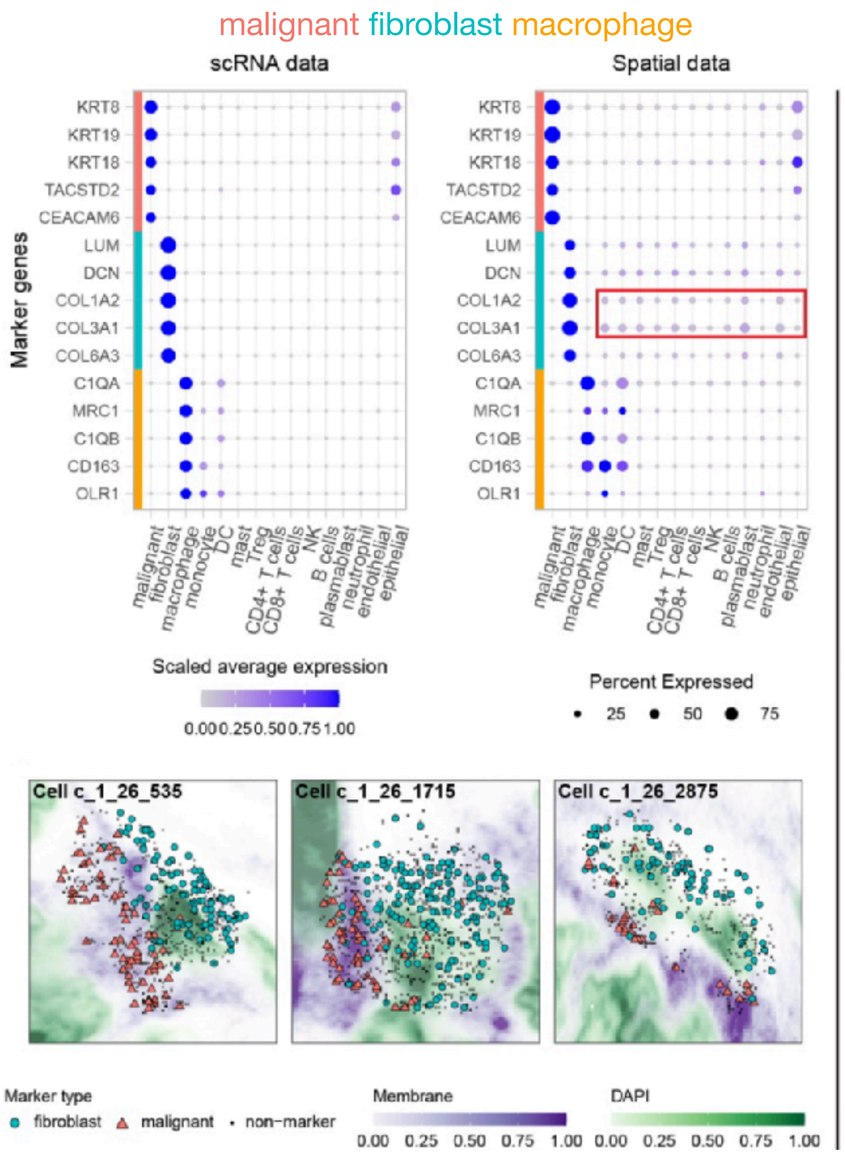
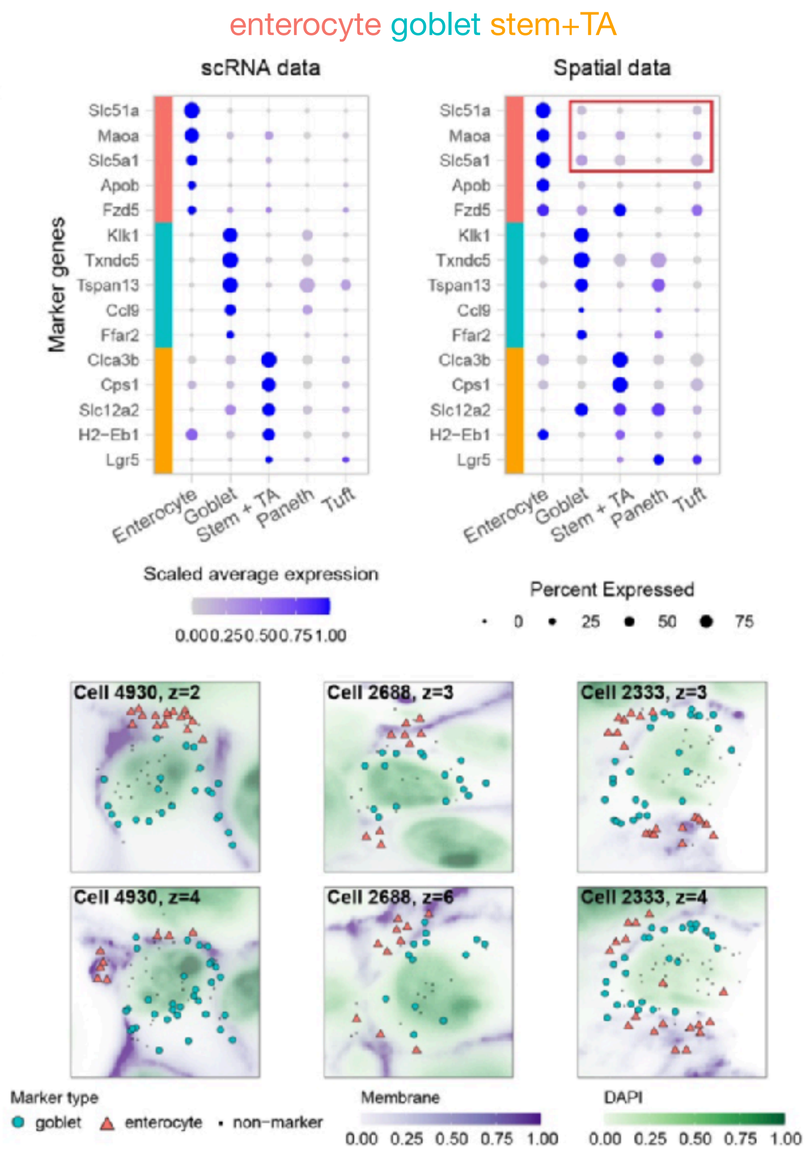


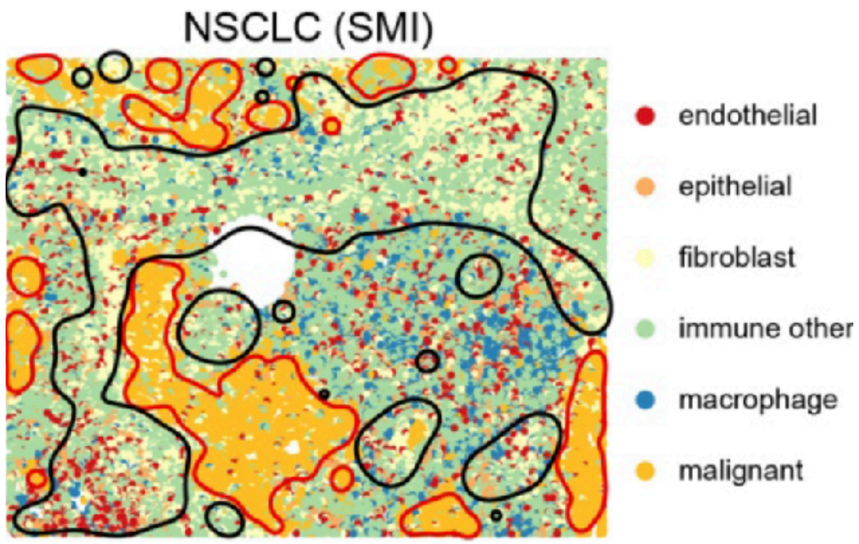


admixtures occur at **upper/lower z-plane**

Mitchel *et al.* (2024), *BioRxiv* 2025.01.02.631135

DE genes between regions reflect compositional differences (not differences in state)

- manual annotation into **tumor** & **stromal** regions



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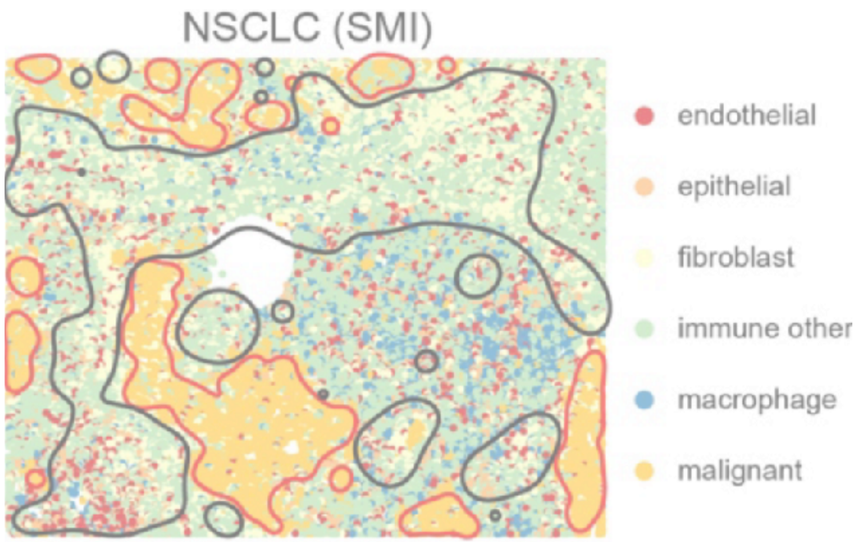

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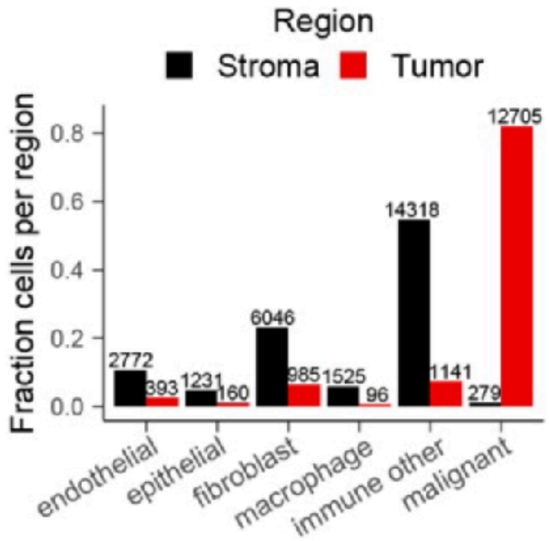

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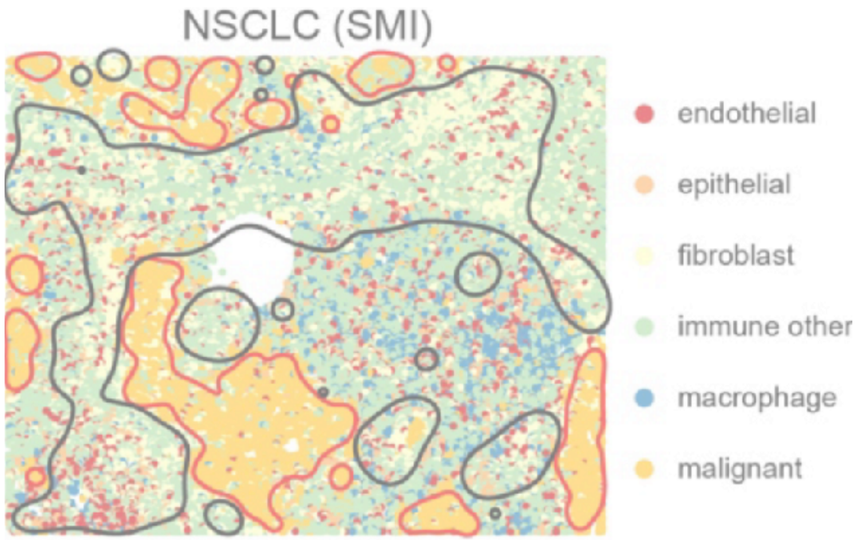

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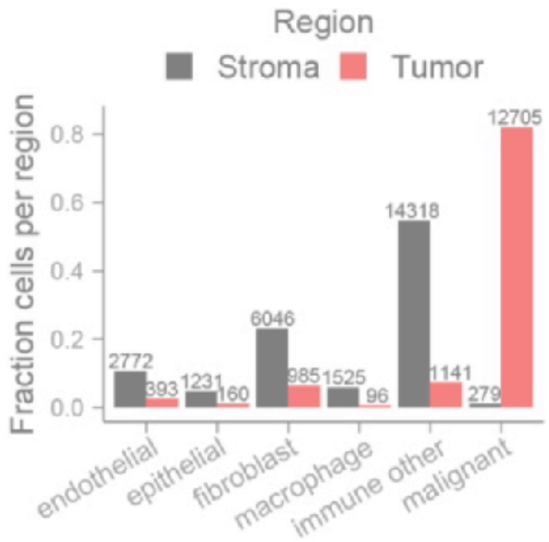
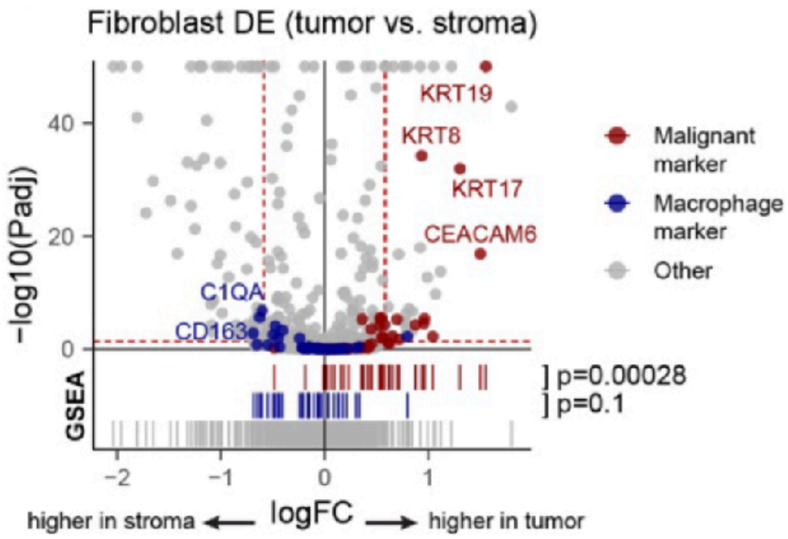


- comparing regions, genes upregulated in fibroblasts are **epithelial markers**



Mitchel *et al.* (2024), *BioRxiv* 2025.01.02.631135

Mitchel *et al.* propose **NMF + CRF clean-up** to mitigate spatial bleeding

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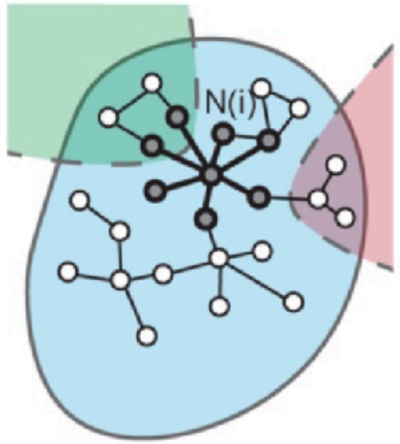

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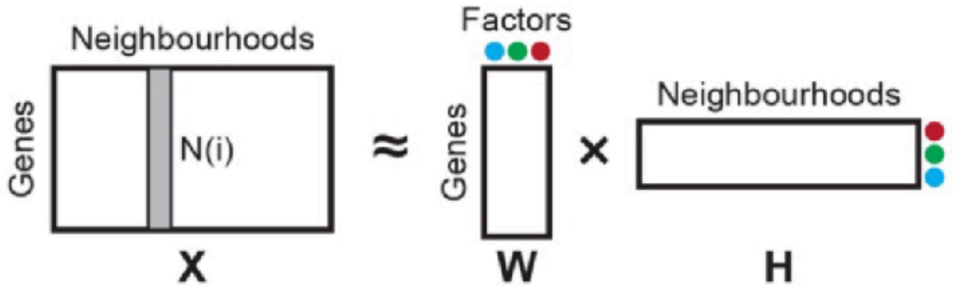

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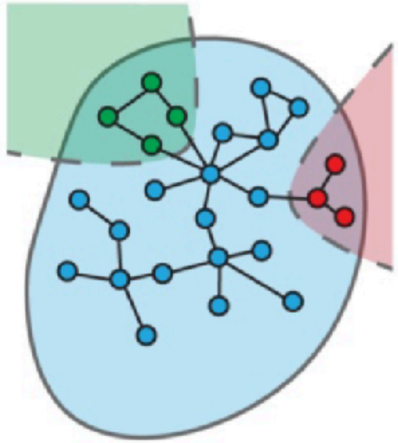

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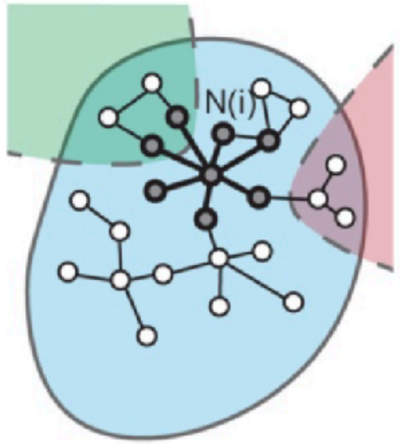

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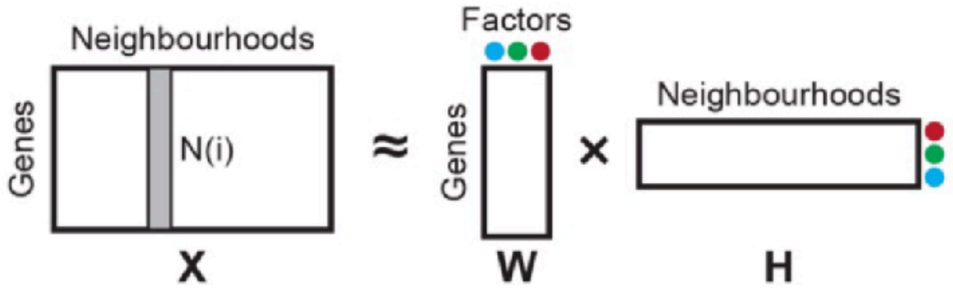

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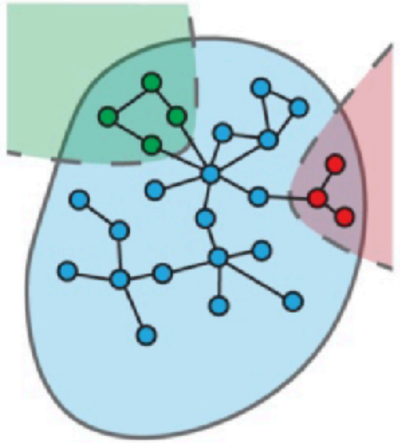

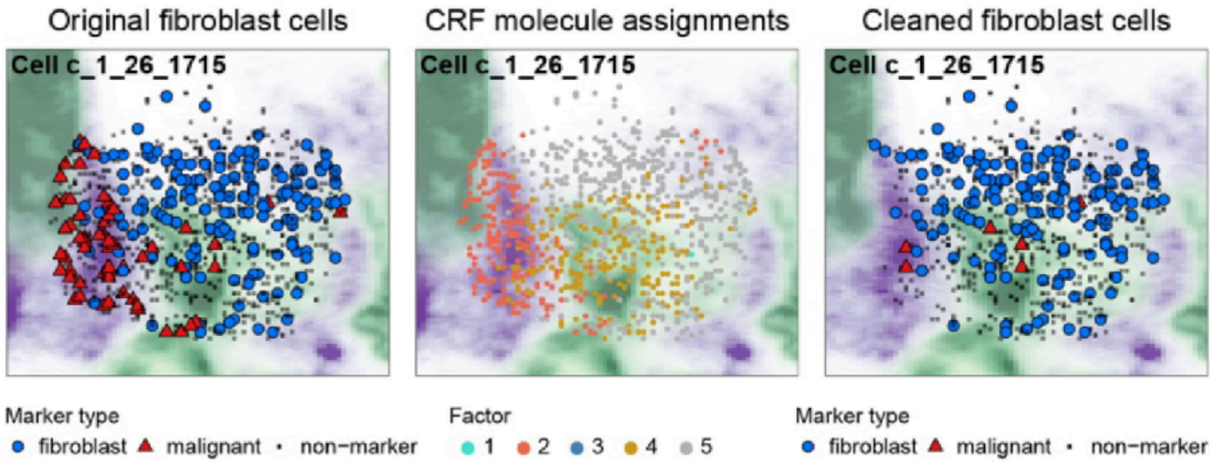

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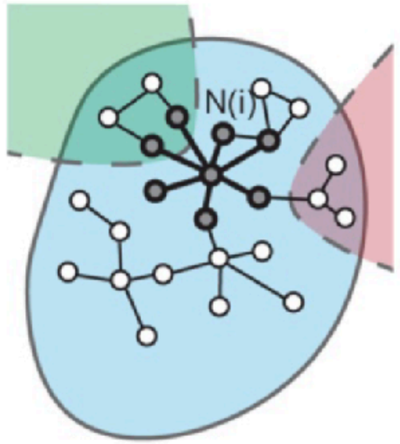

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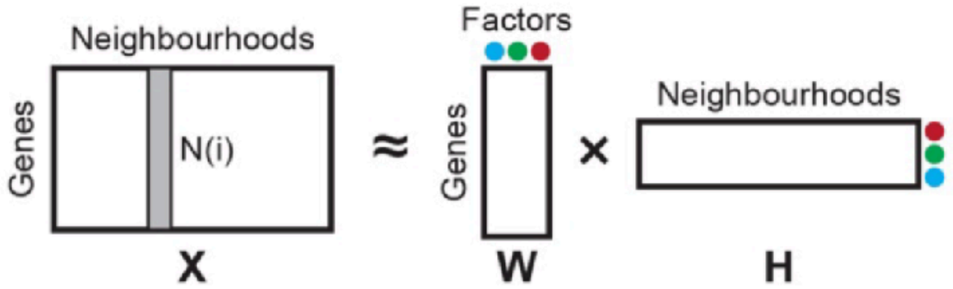

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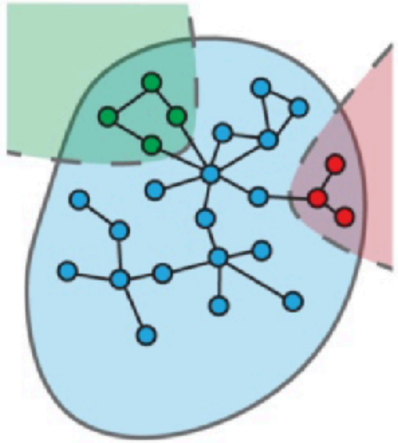
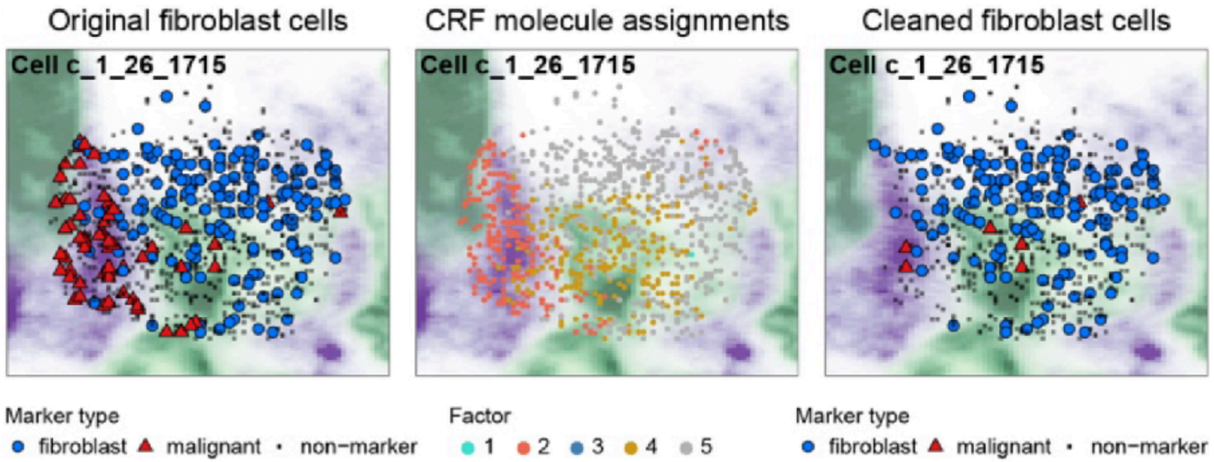


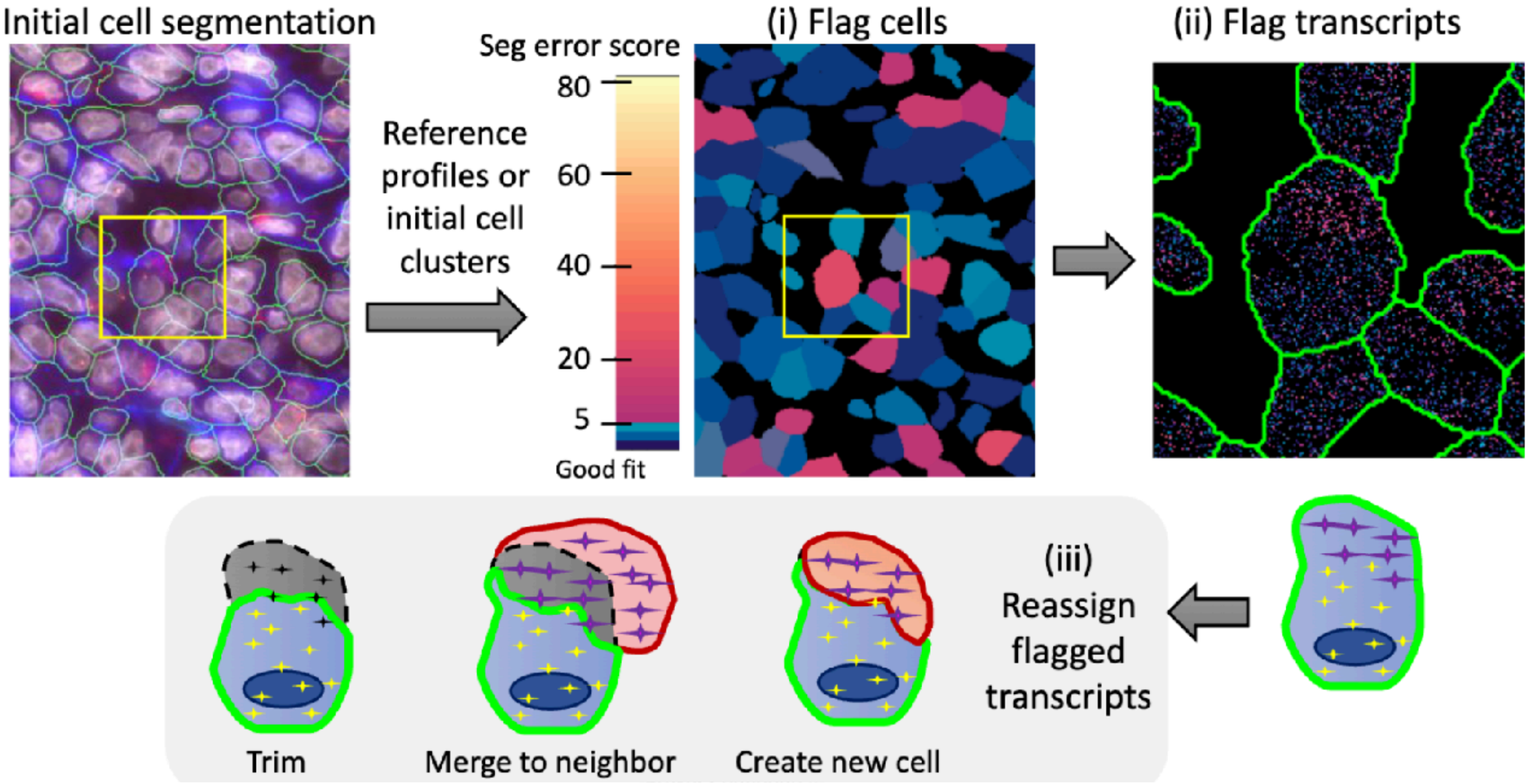
- **subcellular features** can include
  - recurrent **admixture patterns** (e.g., between frequently co-occuring cell types)
  - true **cellular structures** (e.g., ER, nuclei, polarization)



Mitchel *et al.* (2024), *BioRxiv* 2025.01.02.631135

FastReseg uses transcript locations to refine img-based segmentation

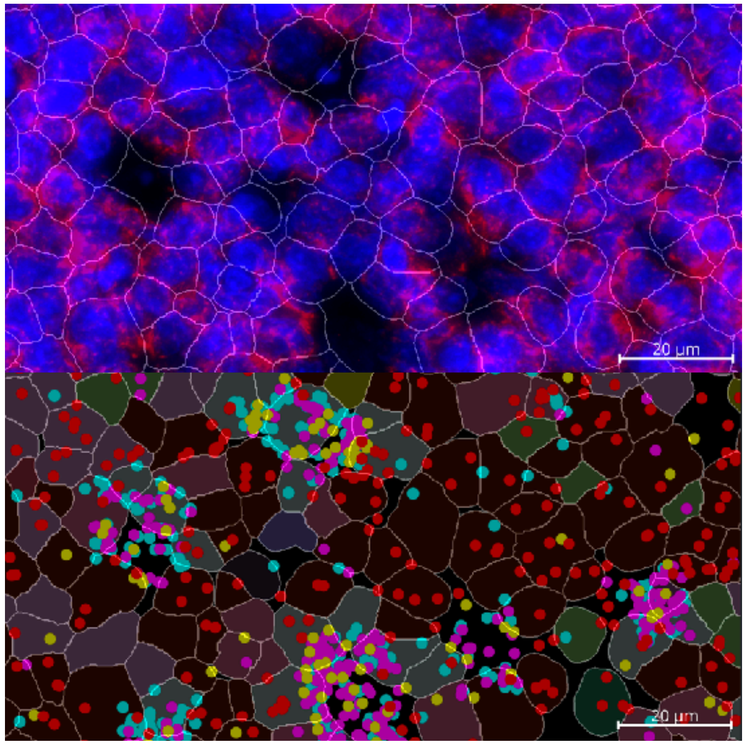
- **transcript scoring**
  based on initial host cell
- **flag spatial doublets**
  as putative segmentation errors
- **flag misassigned transcripts**
  within flagged cells only
- **correct counts** (but not segmentation boundaries)



Wu *et al.* (2024), *BioRxiv* 2024.12.05.627051

FastReseg uses transcript locations to refine img-based segmentation

- flag B cells surrounding black holes
- flag macrophage-related genes
- correct B cell counts & create new macrophages



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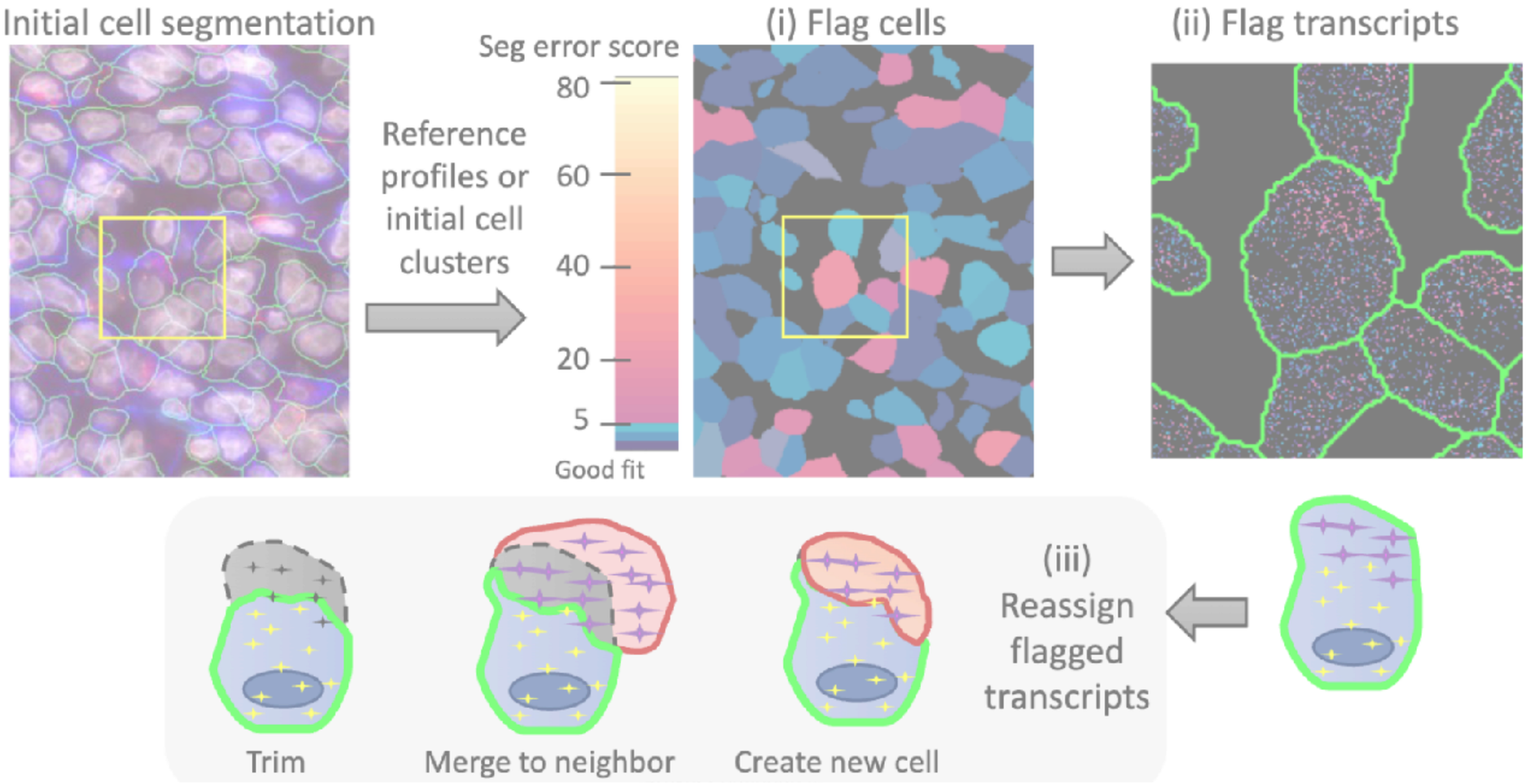
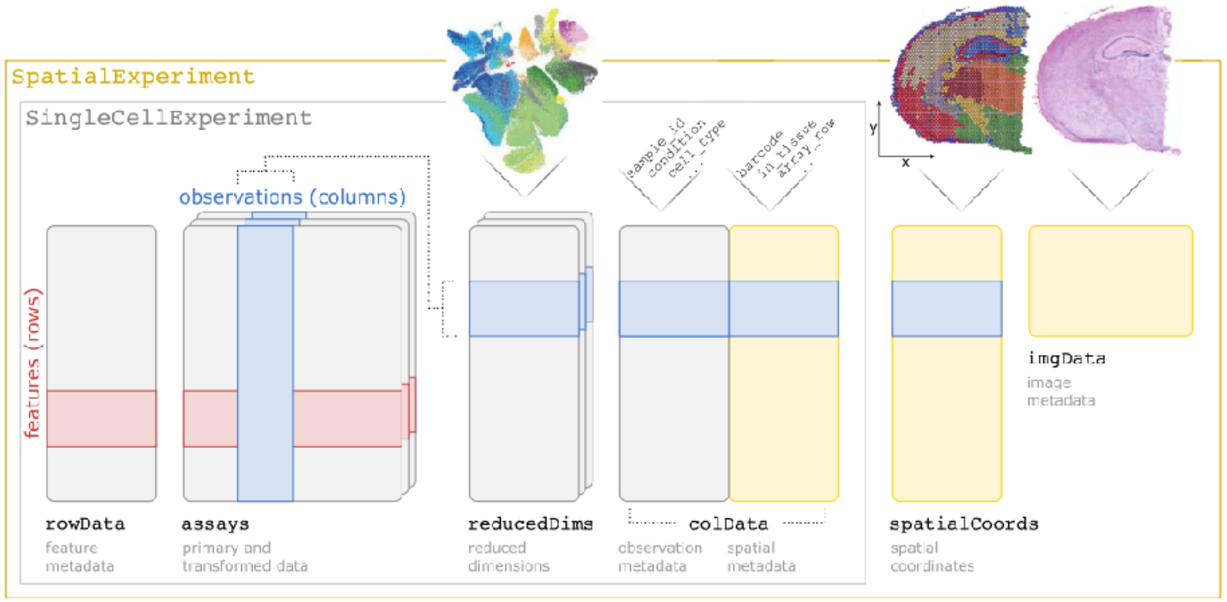


Wu *et al.* (2024), *BioRxiv* 2024.12.05.627051

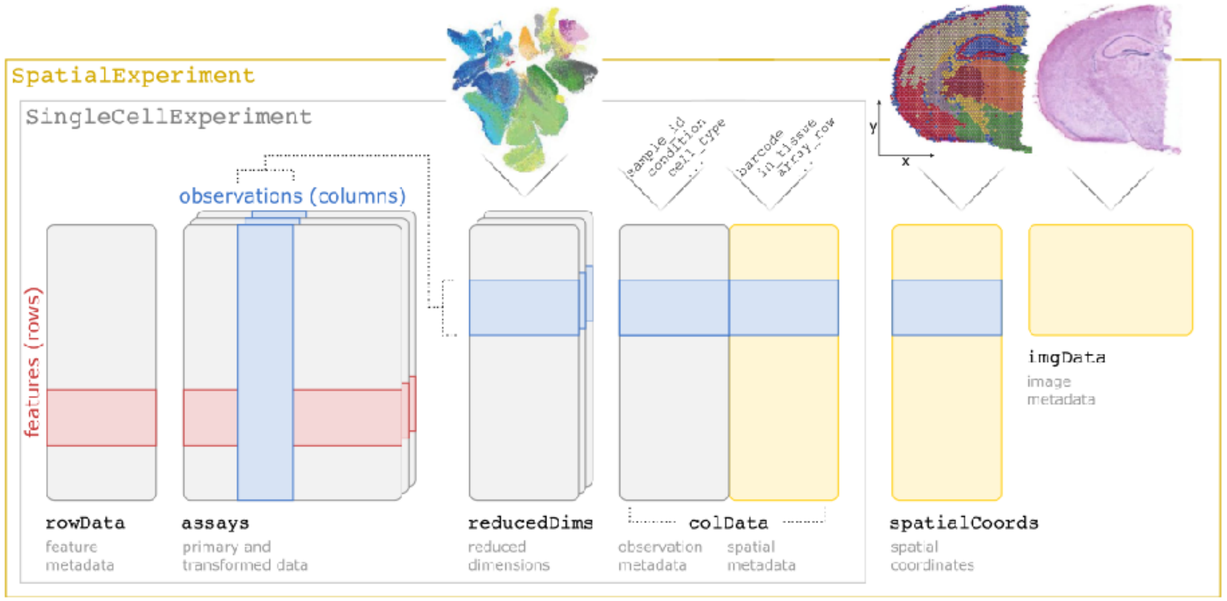
infrastructure for handling img-ST data **in R**



Righelli, Weber, Crowell *et al.* (2022)
Bioinformatics 38(11):3128-3131

SpatialExperiment

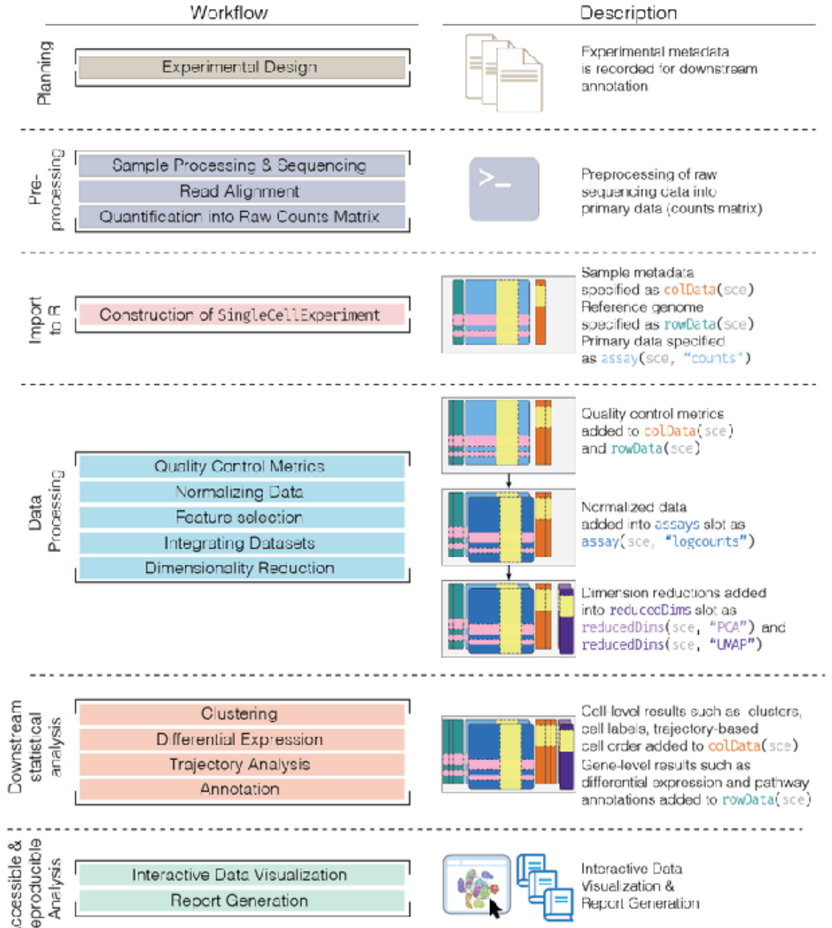
infrastructure for handling img-ST data **in R**



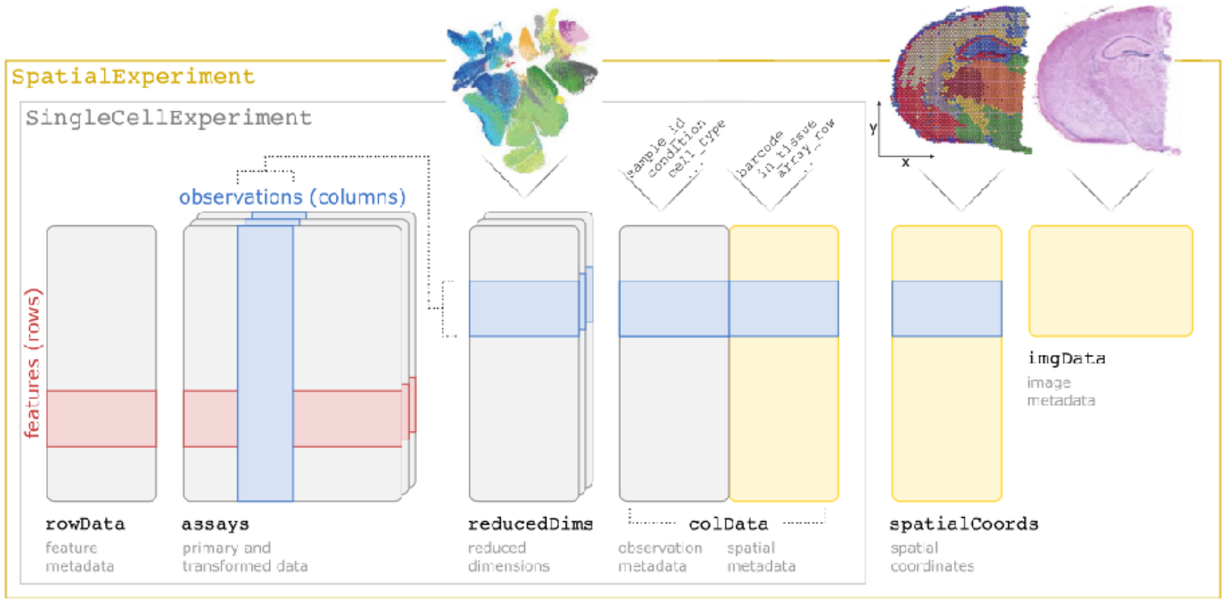
Righelli, Weber, Crowell *et al.* (2022)
Bioinformatics 38(11):3128-3131

SpatialExperiment

Why? Because infrastructure around single-cell data is large!



infrastructure for handling img-ST data **in R**

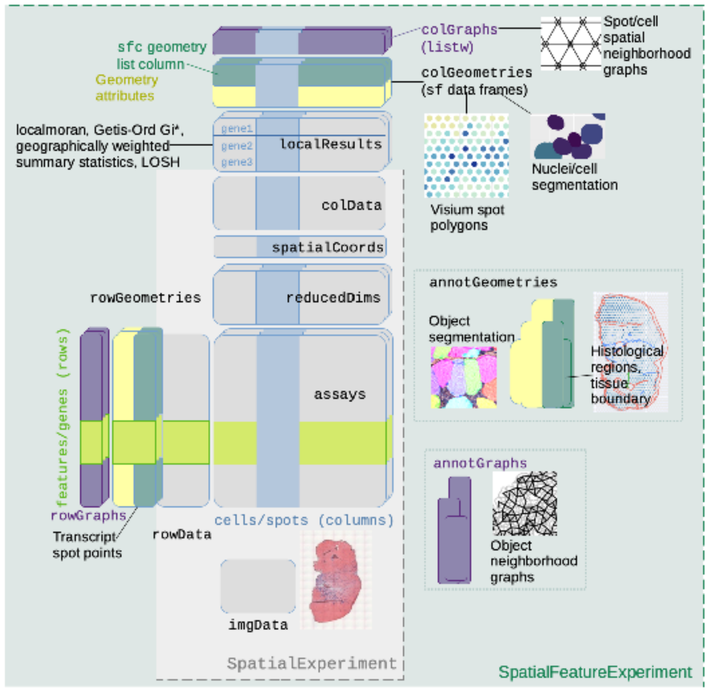


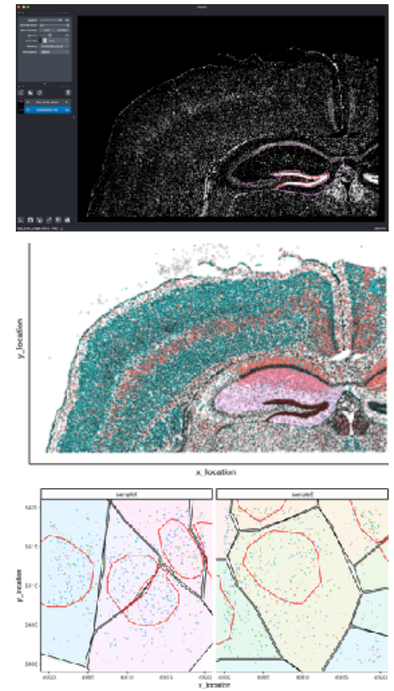
Righelli, Weber, Crowell *et al.* (2022)
Bioinformatics 38(11):3128-3131

SpatialExperiment

Moses *et al.* (2023) *bioRxiv* 2023.07.20.549945

SpatialFeatureExperiment

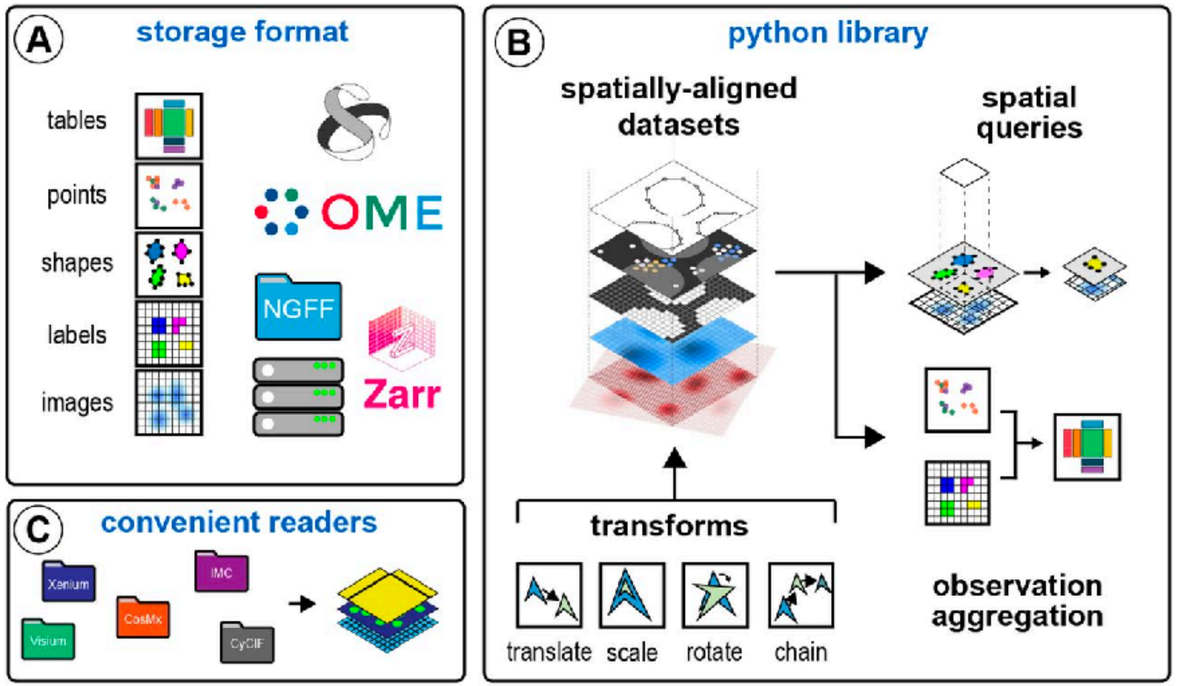




MoleculeExperiment

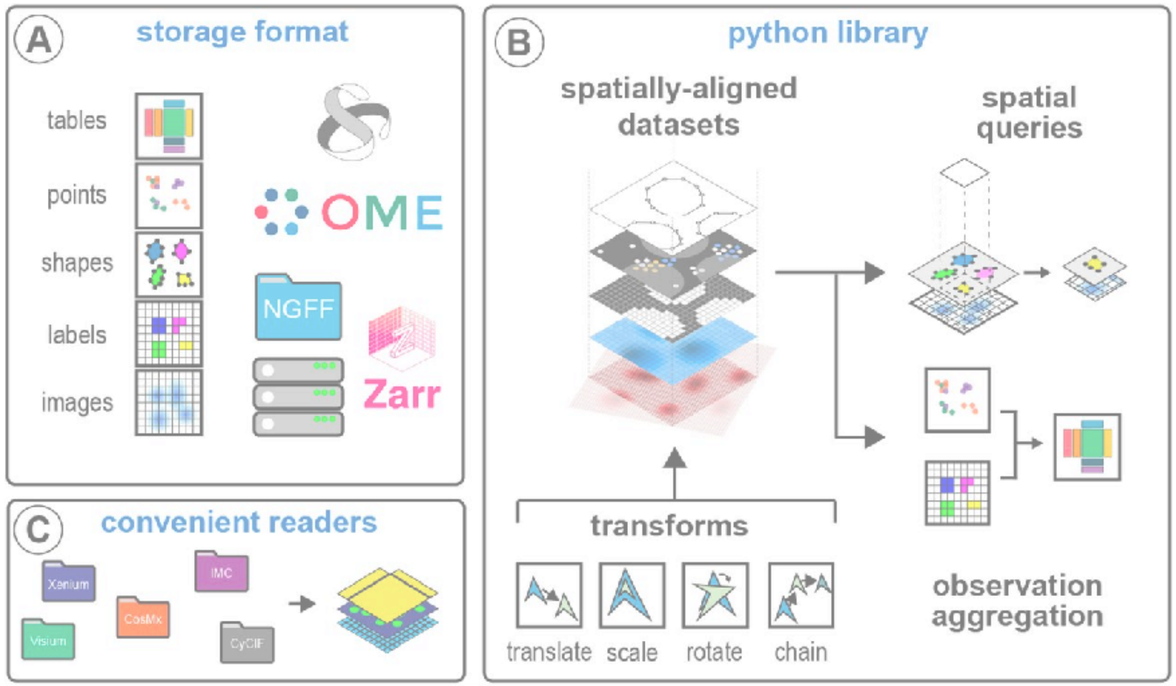
Couto *et al.* (2023) *bioRxiv* 2023.05.16.541040

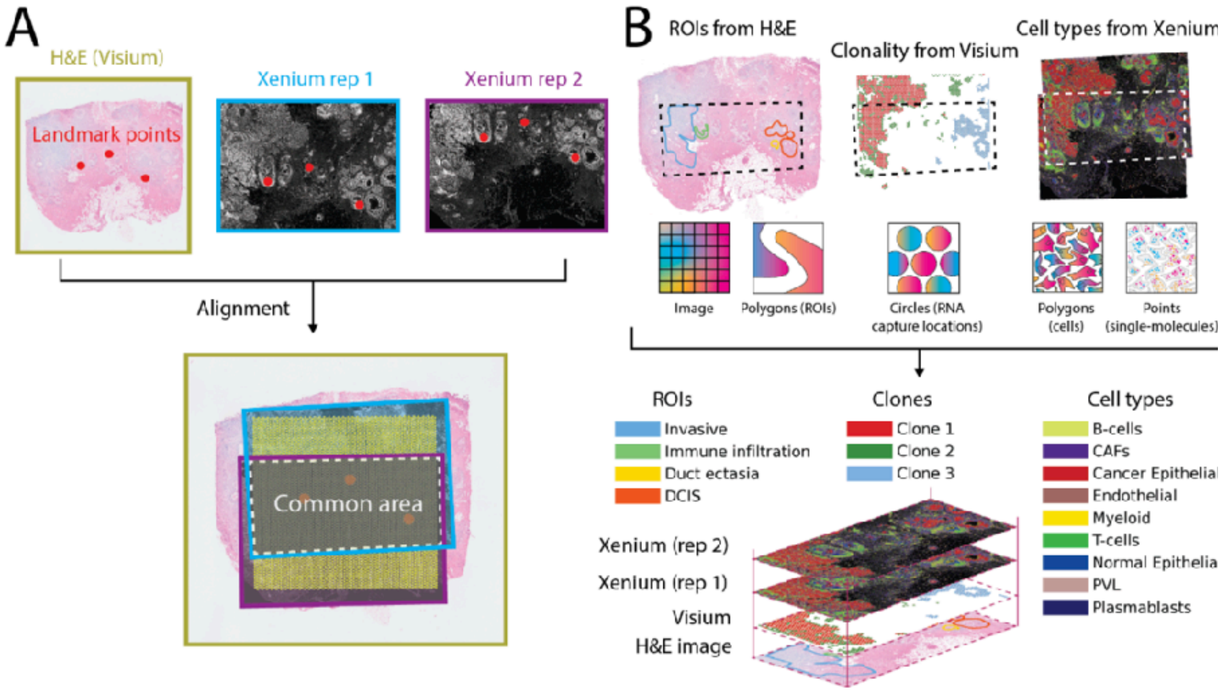
infrastructure for handling img-ST data **in Python**



Marconato *et al.* (2024) *Nature Methods* s41592-024-02212-x

infrastructure for handling img-ST data **in Python**





Marconato *et al.* (2024) *Nature Methods* s41592-024-02212-x

appendix — references & resources

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