Swiss Institute of Bioinformatics

INTRODUCTION TO SPATIAL TRANSCRIPTOMICS DATA ANALYSIS

QC (sequencing-based)

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Which QC do you use for single-cell data?

QC for Visium HD is conceptually the same as single-cell QC – but computed *per High-Definition spot* rather than per cell.

Cell → spots/bins or cell segmentation

Why QC of spatial data?

High-density spots can capture technical artifacts (folds, bubbles, staining issues).

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Poor-quality regions can bias downstream analyses (clustering, spatial DE, trajectory analysis).

QC in Visium HD

Same metrics as single-cell RNA-seq:

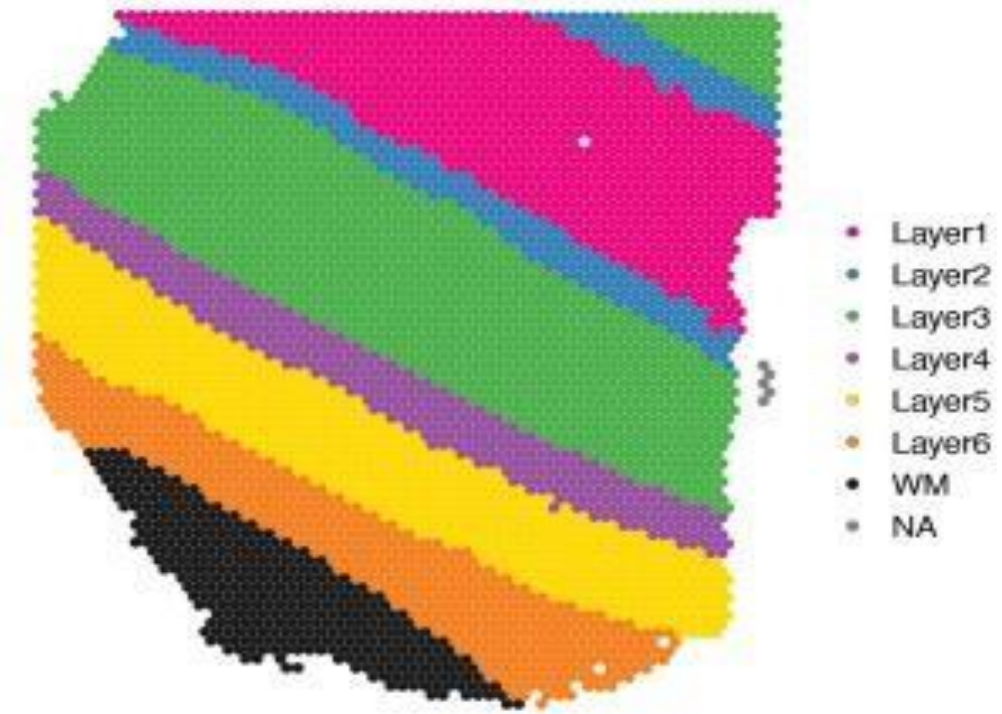
- Total UMIs per spot
- Number of genes per spot
- Mitochondrial fraction

Key difference: unit is HD spot, not single cell.

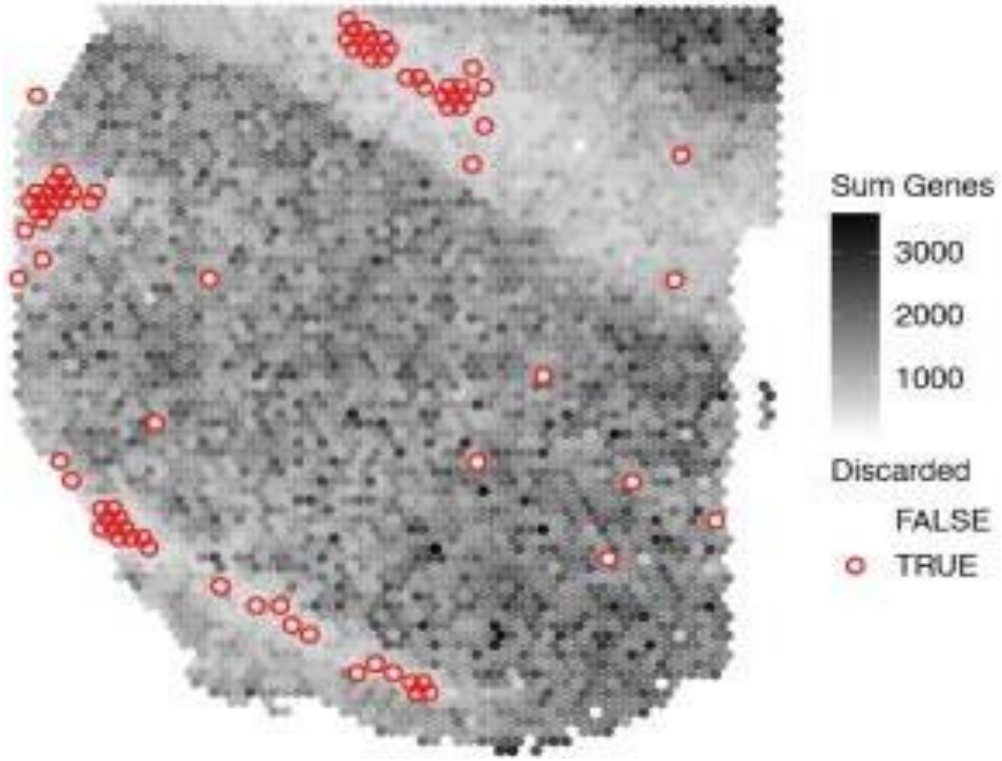
Neighboring spots share microenvironment → spatial artifacts can emerge

Example of spots removal

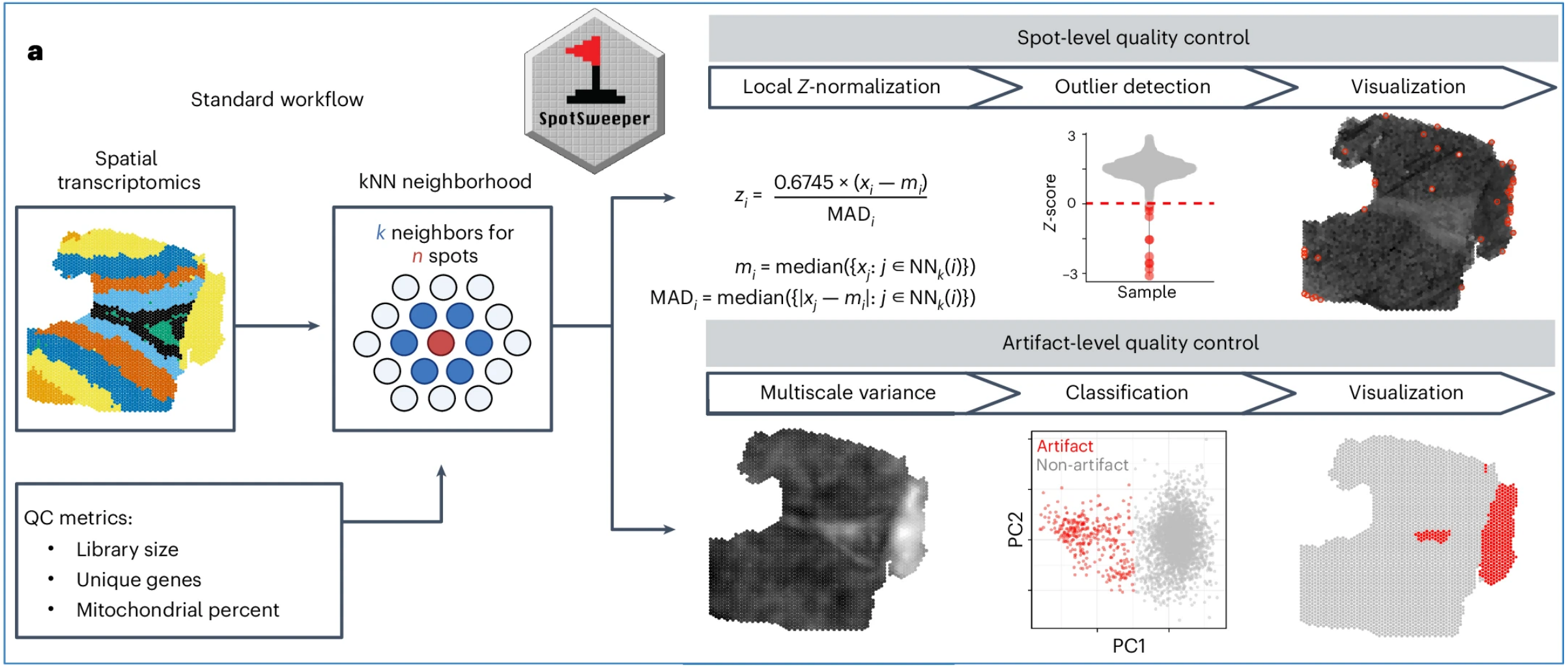
Annotation



Global



SpotSweeper: spatially aware QC



SpotSweeper (spot-level artifacts)

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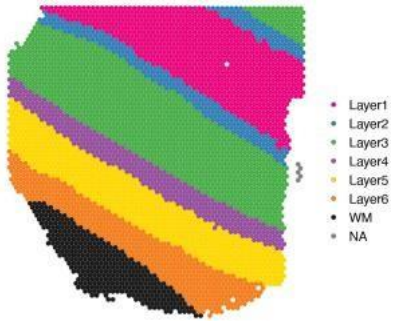

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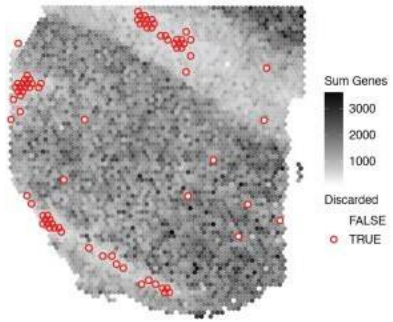

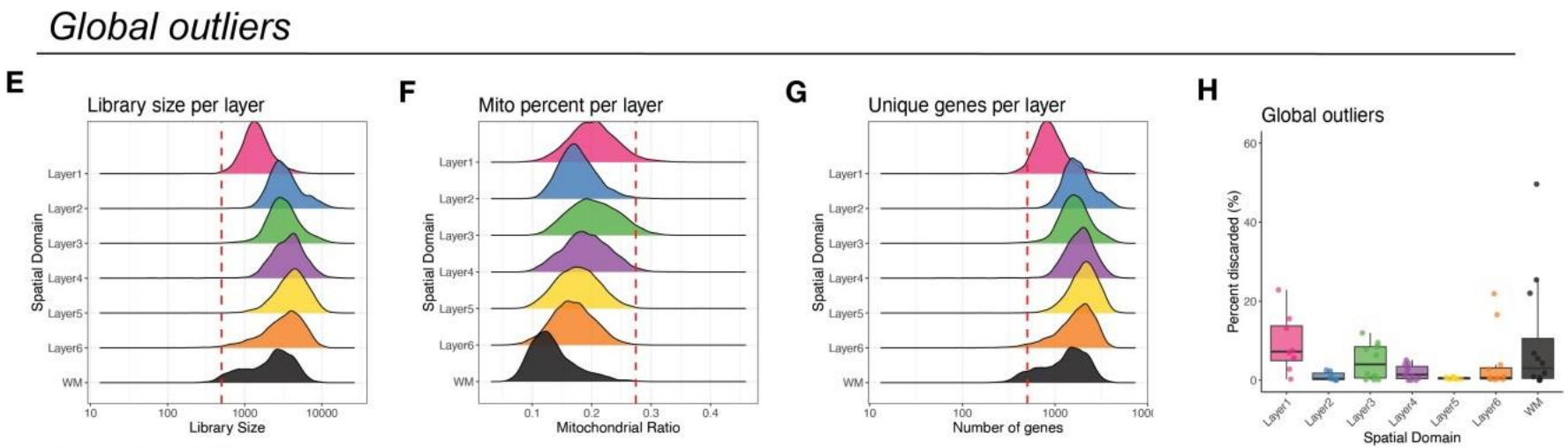

Totty, Hicks, Guo (2024)

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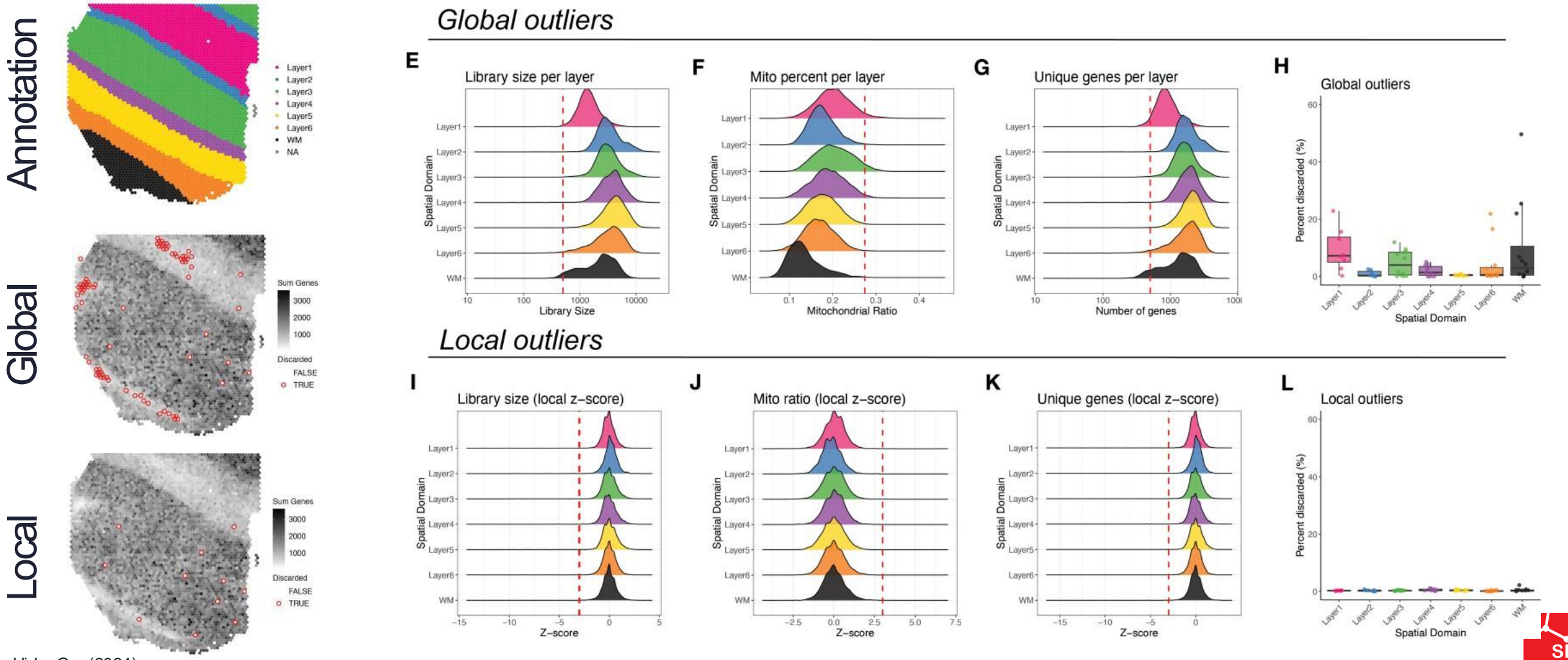

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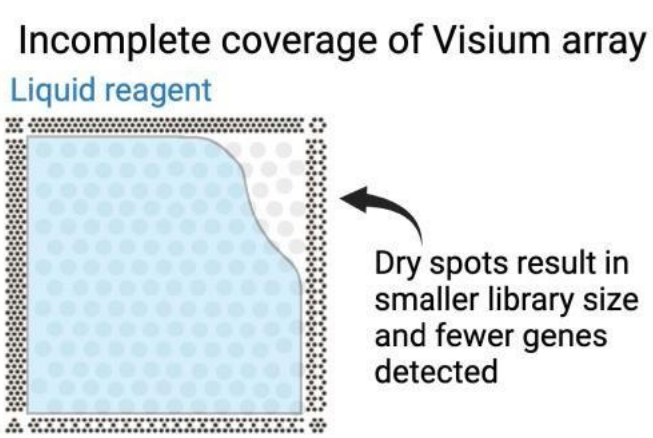
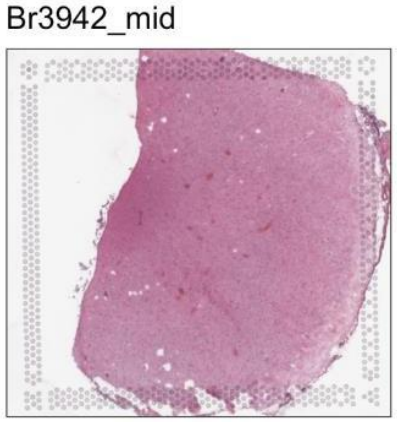
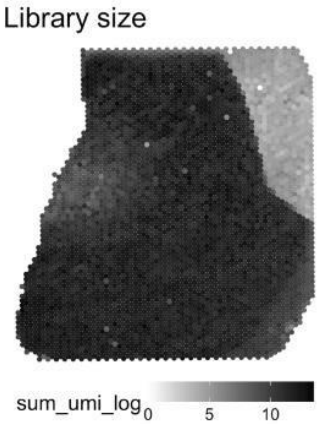
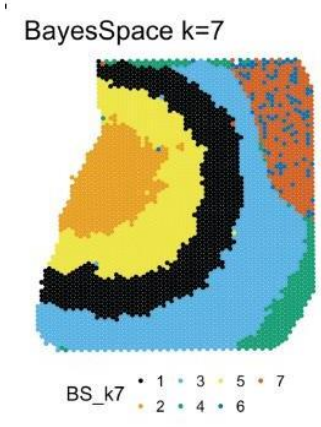
SpotSweeper (region-level artifacts)









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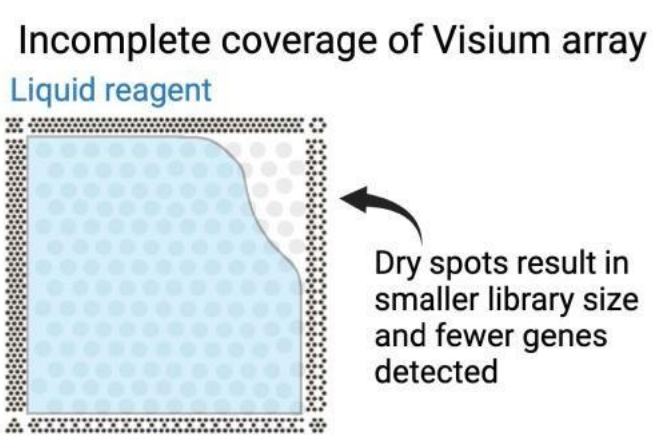

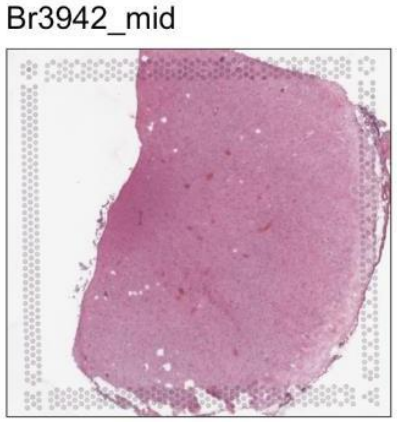

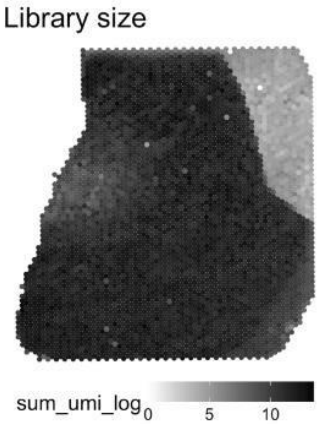

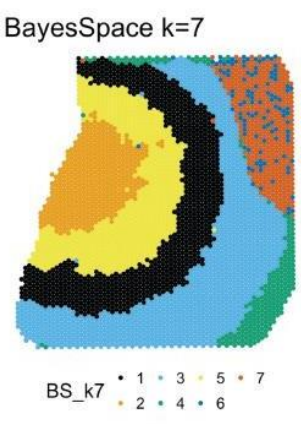

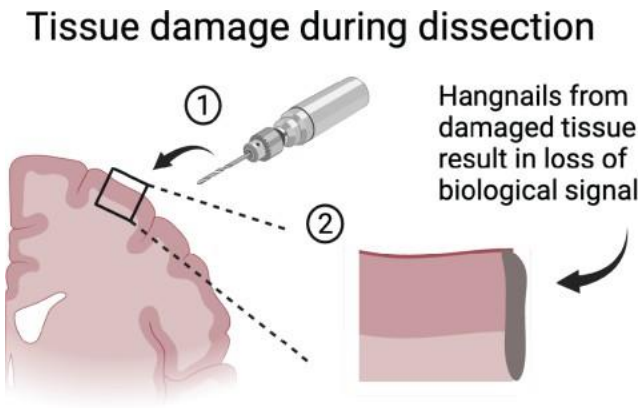

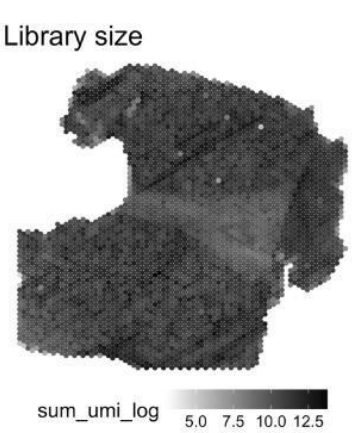

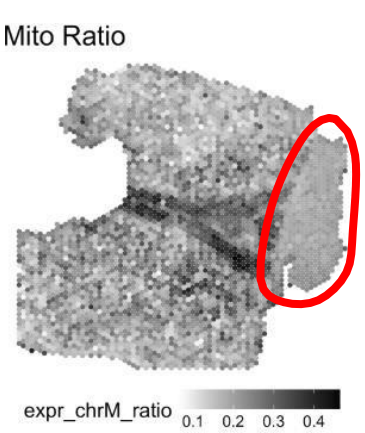

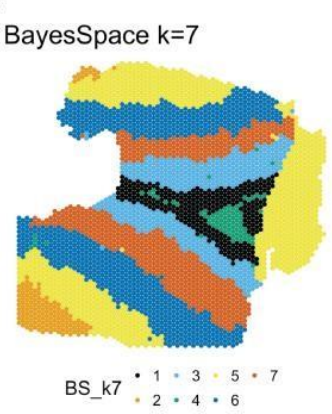

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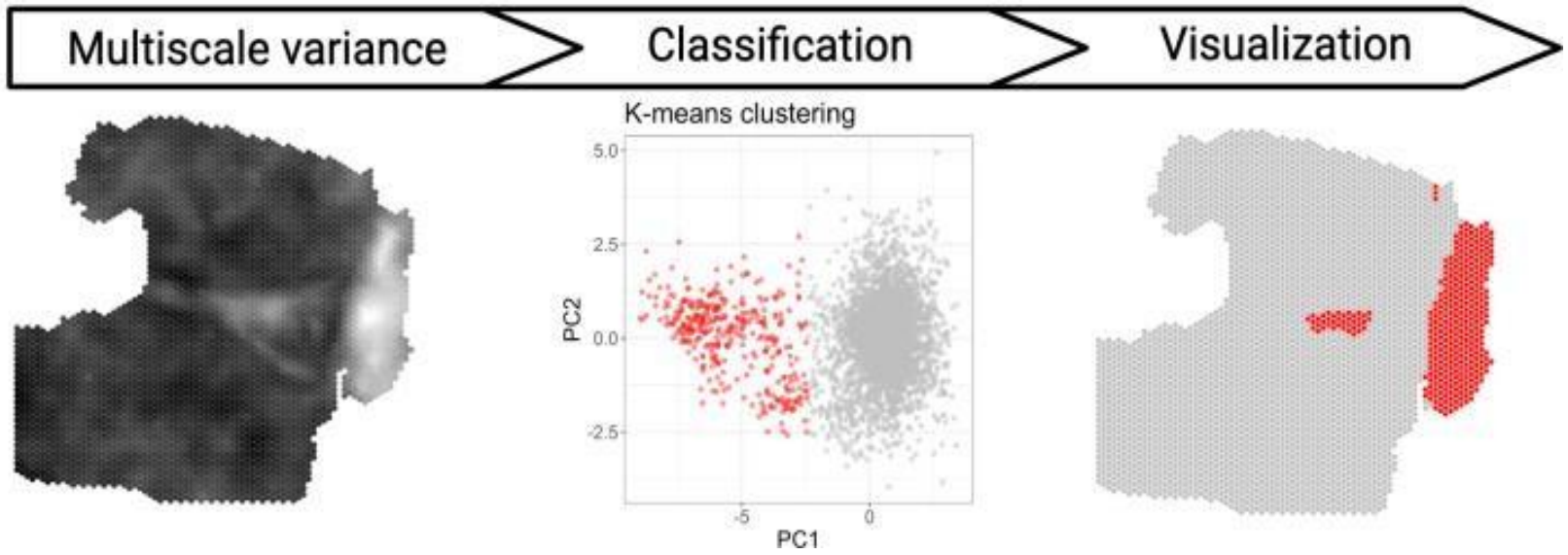
SpotSweeper (region-level artifacts)

1. The **k-NN** for each spot are identified based on the spatial coordinates
2. For each neighborhood size (i.e., scale), local variance of the mitochondrial ratio is calculated and adjusted for a **mean-variance relationship using linear regression**
3. Perform **PCA on the mean-corrected local variances** of all neighborhood sizes
4. Apply **k-means clustering (k=2) in the first two PCs** to identify regional artifacts compared to high-quality tissue



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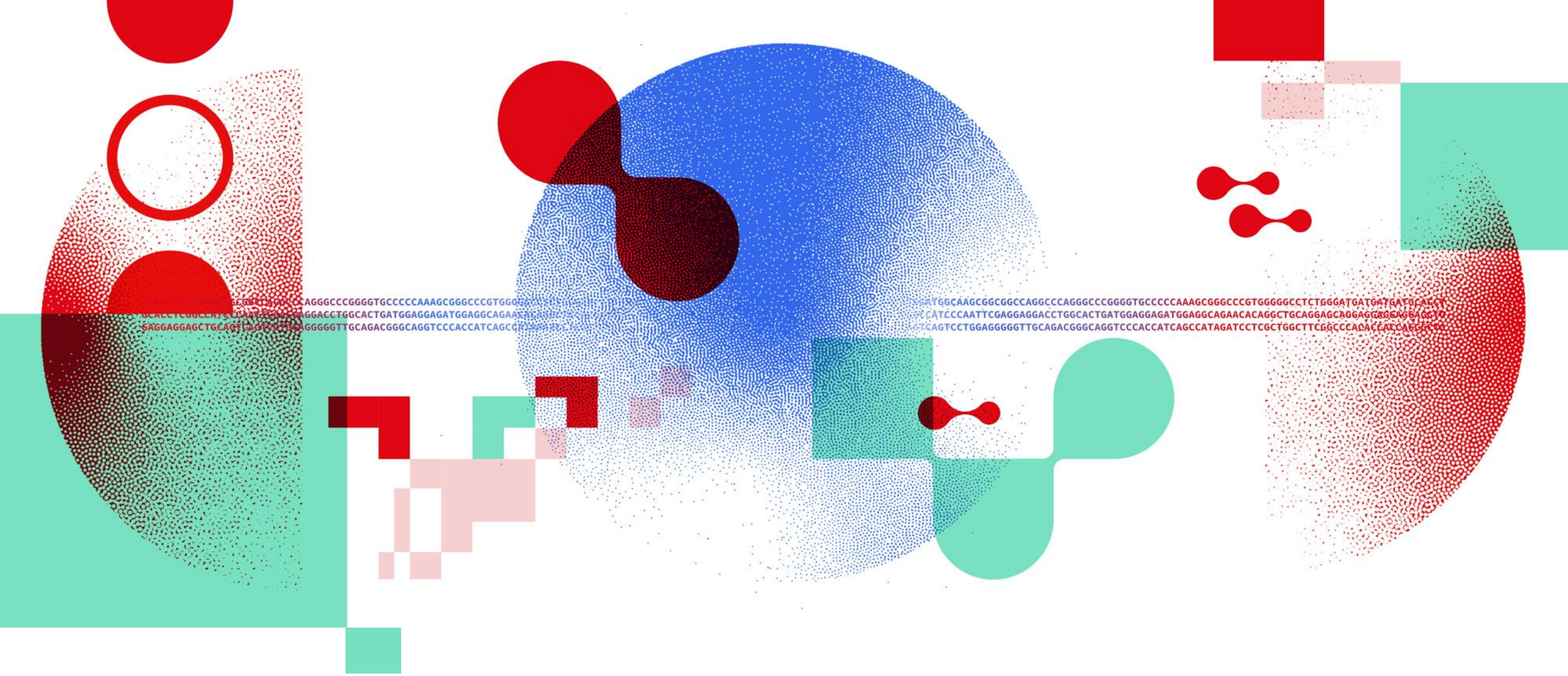
Preserve biological variability while removing technical artifacts: SpotSweeper

Detects **spatially coherent low-quality regions** in Visium HD slides.

Uses **per-spot QC metrics** (mainly mitochondrial fraction) and **spatial neighborhood info**.

Flags artifacts for downstream masking/removal.

Works across **multiple neighborhood scales** to detect both small and large defects.



Thank you

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