Stromal Remodeling — Tutorial Overview

Aim

This notebook applies the **SpatialMMKPNN framework** to explore stromal remodeling in tumor tissues, with a focus on **fibrotic rims in breast cancer FFPE samples**. The workflow identifies and quantifies **ligand–receptor (LR) signaling axes** at stromal–tumor boundaries, highlighting how adhesive and fibrotic motifs contribute to therapeutic resistance.

What This Notebook Shows

- End-to-end pipeline for analyzing stromal remodeling from raw spatial data.

- Explicit construction of stromal rims using FFPE slides.

- Quantification of adhesion and fibrotic signaling (e.g., SPP1, TGFB) at the tumor margin.

- Statistical enrichment analysis for stromal–tumor boundary signaling.

- Robustness checks to confirm stability of rim-based findings.

Key Design Choices (the "why")

- **FFPE data focus:** addresses unique challenges of formalin-fixed slides while preserving reproducibility.

- **Fibrotic rim motif:** spotlights adhesive and fibrotic signals (SPP1, TGFB) known to correlate with stromal barriers.

- **Auditability:** all preprocessing summaries and plots are exported for reproducibility.

- **Console-first results:** enrichment tables and robustness tests are shown directly in the notebook console.

Method (step-by-step)

Preprocessing

- Load FFPE slide data (`.h5ad` or 10x format).

- Normalize counts (CPM → log1p).

- Apply optional gene alias mapping.

- **Output:** `preproc_summary.csv`

Spatial Graph

- Construct k-nearest neighbor graph (k=8) using spot pixel coordinates.

- **Output:** `graph_summary.csv`

Roles (tumor vs stroma)

- Define tumor vs stroma roles using `role_map.csv` or marker-based inference.

- **Output:** `region_summary.csv`

Stromal Rim Definition

- Identify stromal rims by distance transform from tumor–stroma interface.

- Classify spots into rim vs interior.

- **Output:** included in `region_summary.csv`

Edge Accounting, Enrichment, and Robustness

- For each ligand–receptor axis, count stromal→tumor edges and compute rim enrichment.

- Perform robustness checks (rim ±25%, CPM threshold).

- **Results:** printed directly in the console (not saved as CSVs).

Distance-Band Profiles

- Generate WASR-style distance-band analysis for stromal axes.

- **Outputs:**

  - `Plots/ffpe_rims_bands_example.png`

  - `Plots/ffpe_rims_wasr_by_axis.png`

Reproducibility Note

- This tutorial recomputes all outputs end-to-end.

- Only summary files (preprocessing, graph, region definitions) and example plots are written to disk; detailed enrichment results appear in the console.

Results (Stromal Remodeling Summary)

- **SPP1→integrins and TGFB1→TGFBR*:** consistently rim-enriched in stromal regions, forming adhesive and fibrotic rim motifs.
  See enrichment calls printed in the notebook console, and example plots in `Plots/ffpe_rims_wasr_by_axis.png`.

- **VEGFA→KDR:** variable; sometimes rim-enriched, sometimes neutral, reflecting angiogenic remodeling differences across slides.
  See console output for enrichment statistics and `Plots/ffpe_rims_wasr_by_axis.png` *for visualization*.

- **Robustness:** rim enrichment calls for adhesive and fibrotic axes remained stable across thresholds and rim widths.
  Robustness checks are shown in the console.

- **Caveat:** FFPE slides with tissue damage or irregular boundaries produced weaker rim calls.

  Note: Detailed enrichment tables, robustness checks, and distance-band profiles are displayed in the console when running the notebook, not written as CSV files. Only preprocessing summaries and example plots are saved to disk.

Limitations & Recommendations

- **Role inference variability:** marker-derived tumor/stroma boundaries may be imprecise; curated annotations recommended.

- **Geometry dependence:** rim definitions can shift with tissue quality (especially in FFPE).

- **Slide heterogeneity:** VEGFA enrichment varied, suggesting biological and technical variability.

- **FFPE artifacts:** RNA quality and morphology can influence robustness of enrichment calls.

Recommendations:

- Use curated role annotations when possible.

- Interpret rim enrichments as **relative** (slide-specific) rather than absolute measures.

- Validate findings across multiple FFPE slides.

- Explicitly report robustness checks.

Troubleshooting

- **Weak rim definition:** adjust blur sigma or distance thresholds.

- **Few stromal spots:** provide curated role map to stabilize rim.

- **Counts too low:** relax expression threshold; note change.

Interpretation

This application highlights how **stromal remodeling creates adhesive and fibrotic barriers** at tumor rims. Enrichment of SPP1 and TGFB signaling suggests a conserved stromal motif linked to therapeutic resistance.

By combining **statistical rigor** (enrichment tests, robustness checks) and **interpretability** (explicit LR edges, pathway attribution), this tutorial shows how SpatialMMKPNN can dissect the **fibrotic interface of tumors** in FFPE data.