Virtual RNA Inference of Xenium ST from H&E @ Dartmouth and Cedars-Sinai

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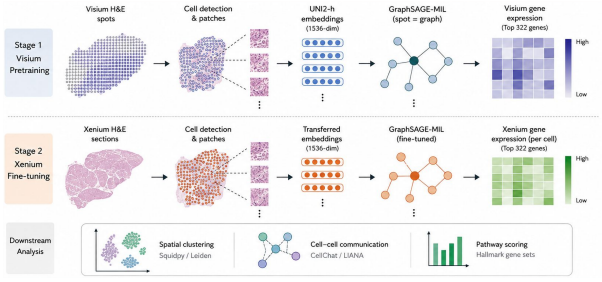
Abstract

- **Motivation**: Spatial transcriptomics provides valuable molecular context but remains costly and low-throughput, limiting routine clinical use.
- **Approach**: We developed a two-stage Virtual RNA Inference (VRI) framework to predict single-cell spatial gene expression directly from routine H&E imaging.
- **Visium pretraining**: GraphSAGE-MIL outperformed MLP (0.291 vs. 0.276 Spearman).
- **Xenium fine-tuning**: Selective transfer improved over random initialization (0.1908 vs. 0.1894 Pearson).
- **Outcome**: Predicted profiles recovered spatially and biologically coherent tissue structure.

Introduction

- **Problem**: Spatial transcriptomics is expensive and labor-intensive, limiting clinical scalability while H&E is inexpensive and routinely collected.
- **Goal**: Infer spatial gene expression from H&E alone in colorectal cancer, a leading cause of cancer death.
- **Novelty**: Extends VRI to CRC and benchmarks predictions against Xenium at single-cell resolution, rather than spot-level Visium alone.
- **Hypothesis**: AI-inferred spatial transcriptomic profiles from H&E will retain sufficient biological and spatial signal to support downstream analyses and associate with clinical outcomes, even without direct Xenium profiling.

Methods



Methods pipeline: From paired H&E/spatial transcriptomics data to per-cell gene expression prediction and downstream analysis.

Methods

Data: 24 Xenium sections / 8 patients (322-gene panel) and 300K Visium spots / 40 CRC specimens from DHMC and Cedars-Sinai.

Visium Pretraining:
- Specimen-level spatial graphs with 2-layer GraphSAGE + gated-attention Multiple Instance Learning (MIL)
- Compared with a non-spatial MLP baseline
- MSE + Pearson loss with 5-fold patient-level CV

Xenium Fine-Tuning:
- Transferred selected components from a Visium-pretrained MLP
- Used a skip connection + differential learning rate
- GraphSAGE backbone randomly initialized
- Trained on 4 DHMC patients; validated on 4 independent held-out DHMC patients

Downstream:
- Cell typing: pyUCell + scANVI
- Spatial clustering: Squidpy/Leiden
- Biology: pathway scoring + LIANA cell-cell communication

Results

- **Visium pretraining**: GraphSAGE-MIL outperformed the non-spatial MLP across all 5 folds (mean Spearman: 0.3126 vs. 0.3056).
- **Xenium fine-tuning**: Visium-pretrained transfer modestly outperformed random initialization (Pearson: 0.1908 vs. 0.1894; Spearman: 0.1771 vs. 0.1750).

Model	Fold 0	Fold 1	Fold 2	Fold 3	Fold 4	Mean CV
GraphSAGE-MIL	0.3108	0.3364	0.2905	0.3130	0.3138	0.3126
MLP	0.3051	0.3284	0.2834	0.3056	0.3057	0.3056

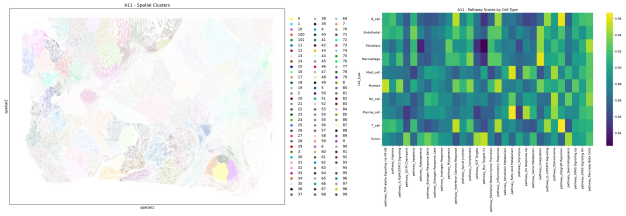
Table 1: Visium 5-Fold Cross-Validation Performance

Model	Mean Pearson	Mean Spearman
Random initialization (baseline)	0.1894	0.1750
Visium-pretrained transfer (skip connection + differential LR)	0.1908	0.1771

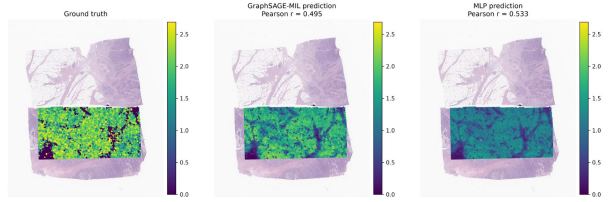
Table 2: Xenium Transfer Learning Performance

Results

- **Spatial recovery**: Per-cell predictions reproduced ground-truth spatial expression patterns in held-out Visium and unseen Xenium tissue.
- **Biological coherence**: Predicted cell types and pathways matched expected tissue biology, with tumor cells enriched for proliferation and T cells for immune pathways.



(Spatial clusters + pathway heatmap): Leiden spatial clustering and Hallmark pathway scores by cell type in specimen A11, showing spatially coherent tissue domains and cell-type-specific pathway activity.



(MKI67 prediction): Predicted vs. measured MKI67 expression in a held-out Visium specimen; both GraphSAGE-MIL and MLP recover the spatial expression pattern seen in ground truth.

Conclusion

- **Spatial modeling improved prediction:** GraphSAGE-MIL outperformed the non-spatial MLP across Visium folds, while Visium pre-training improved Xenium fine-tuning over random initialization.
- **Recovered biological states:** Predicted expression captured coherent tissue organization, with tumor cells highest for proliferation and T cells highest for immune pathways, consistent with CRC biology.
- **Generalized to unseen tissue:** The pipeline performed on held-out Xenium sections and cross-institutional evaluation, supporting meaningful transcriptomic signal in H&E morphology.
- **Scalable biomarker discovery:** H&E-based VRI offers a low-cost approach to spatial biomarker discovery, extending molecular insights beyond samples that undergo direct spatial profiling.

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