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Single Cell RNA-Sequencing of Superficial Non-Muscle Invasive Bladder Cancer (NMIBC): Proof of Principle and Early Analysis

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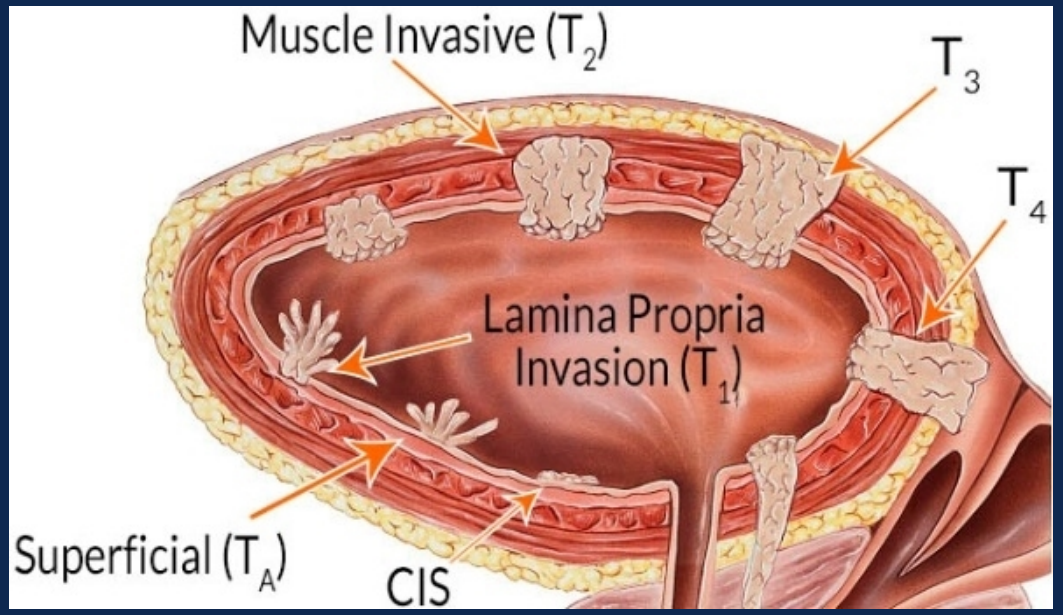
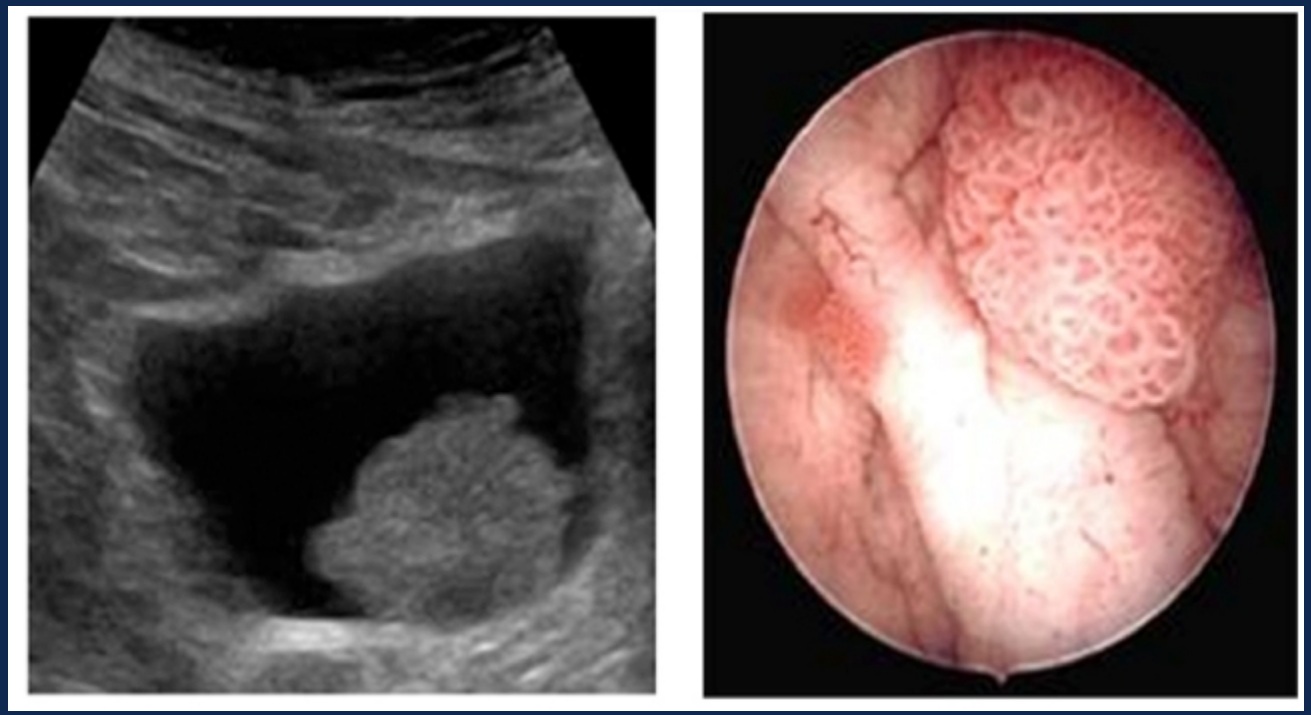
Urothelial Cancer





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NMIBC

- Tumor recurrence
- Treatment resistance
- Tumor progression
- Tumor monitoring
- Specific aim – to demonstrate feasibility of single cell RNA seq of NMIBC cells to allow for further evaluation of tumor heterogeneity at a higher level of resolution.

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Rationale for scRNA-seq in urothelial carcinoma

- Ability to identify key subpopulations of cells of interest.
- Can distinguish transcriptomes of key subclonal populations or infiltrating immune cell subtypes.
- Can find expression signatures in a subpopulation of cells that would normally be swamped out by bulk RNA-seq.

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Materials and Methods

- Tumor samples N =5
  - 3 LGTa lesions
  - 2 HGTa lesions
- Mechanical disaggregation and preparation for 10X Chromium system
- Visual representation via T-distributed stochastic neighbor embedding
- Comparison of several metabolic parameters

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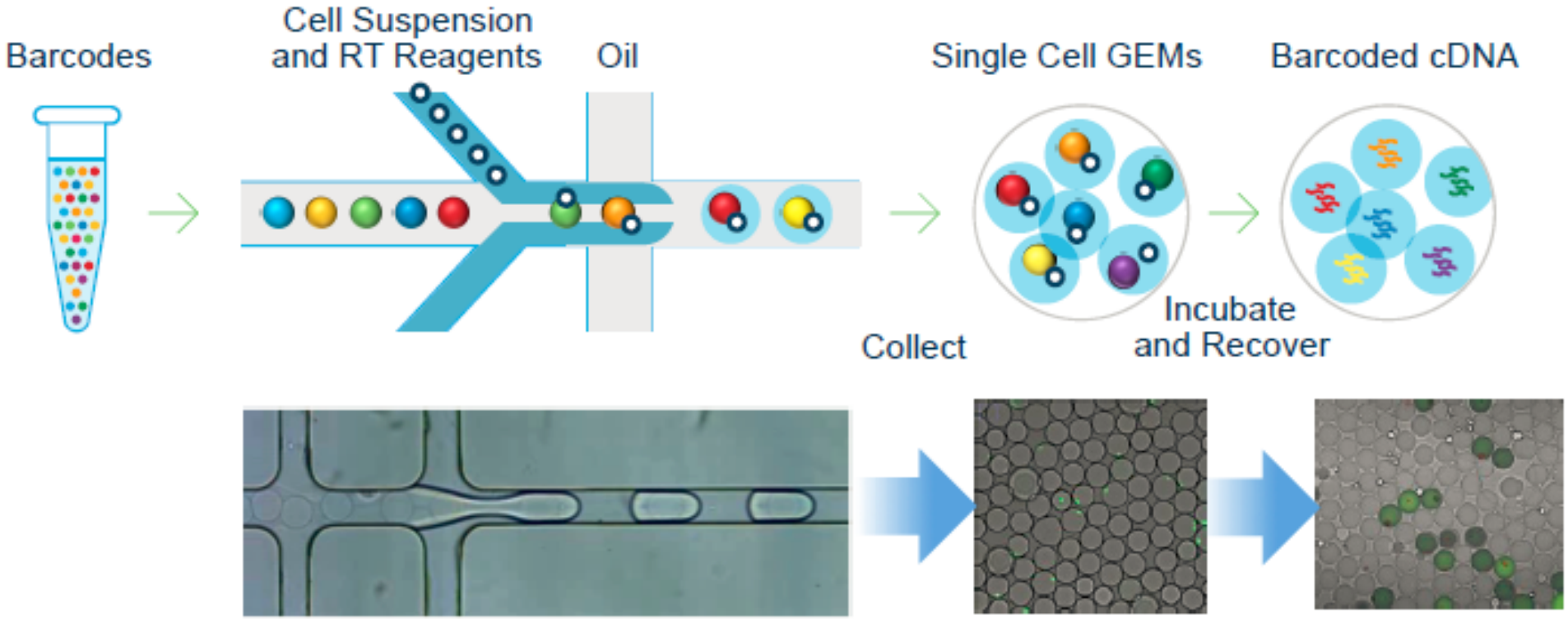
scRNA-seq library prep on the 10X Chromium

- Single cells are embedded in emulsion gel beads.
- All RNA (cDNA) within a bead are tagged with the same barcode.
- Oligos from all cells are then pooled for bulk sequencing.

Overview of the 10X GEM Procedure

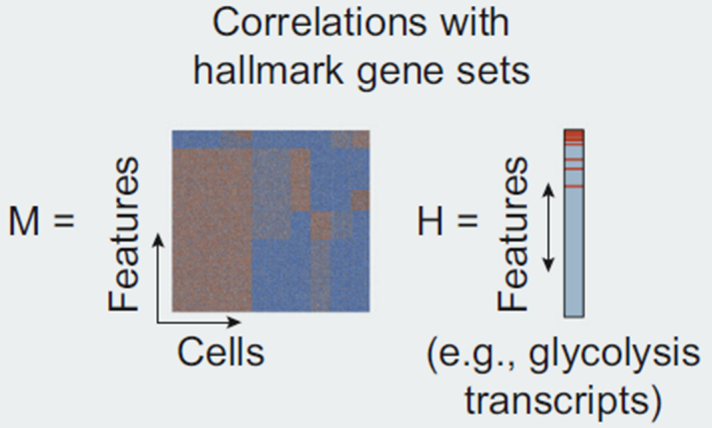


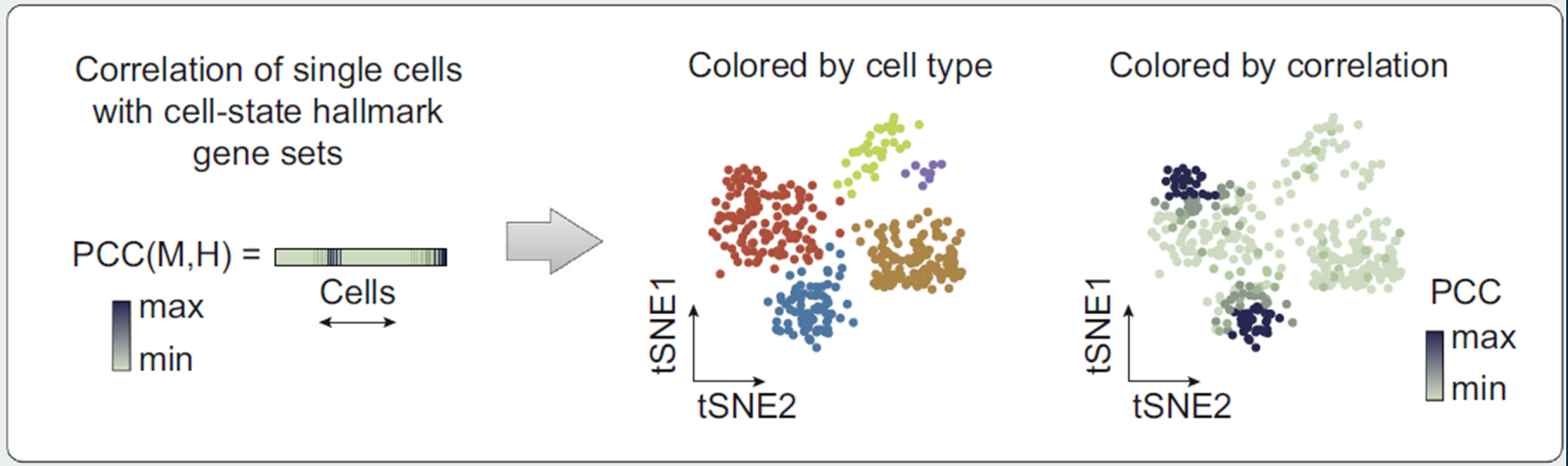
- High GEM fill ratio (~90% of droplets contain beads)
- Poisson loading of cells into GEMs
- Beads dissolve for efficient, liquid phase biochemistry

Cell Identification and Stratification

Classifying cell type-specific states in single cells

Annotations associated with specific cell states, such as gene sets of known hallmarks, can be used to identify cell type-specific states in single-cell data. Measuring the Pearson correlation coefficient (PCC) of single cells with known hallmarks can be used to segment cells based on their epigenetic-metabolic states.





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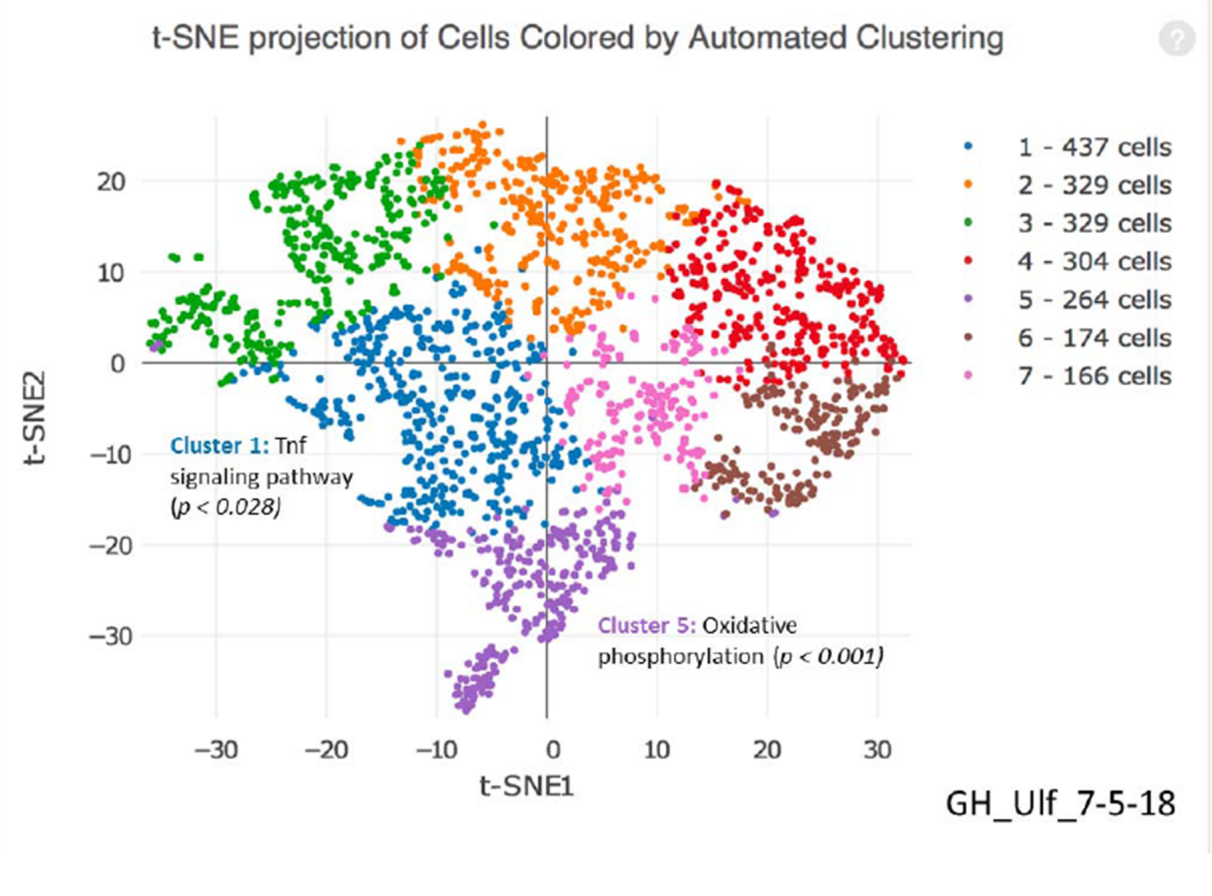
T-distributed stochastic neighbor embedding in a low grade Ta UCC tumor



$$p_{j|i} = \frac{\exp(-\|\mathbf{x}_i - \mathbf{x}_j\|^2 / 2\sigma_i^2)}{\sum_{k \neq i} \exp(-\|\mathbf{x}_i - \mathbf{x}_k\|^2 / 2\sigma_i^2)},$$

A machine learning algorithm for visualization
Embeds high –dimensional data for visualization in Low dimensional space (2-3 dimensions)

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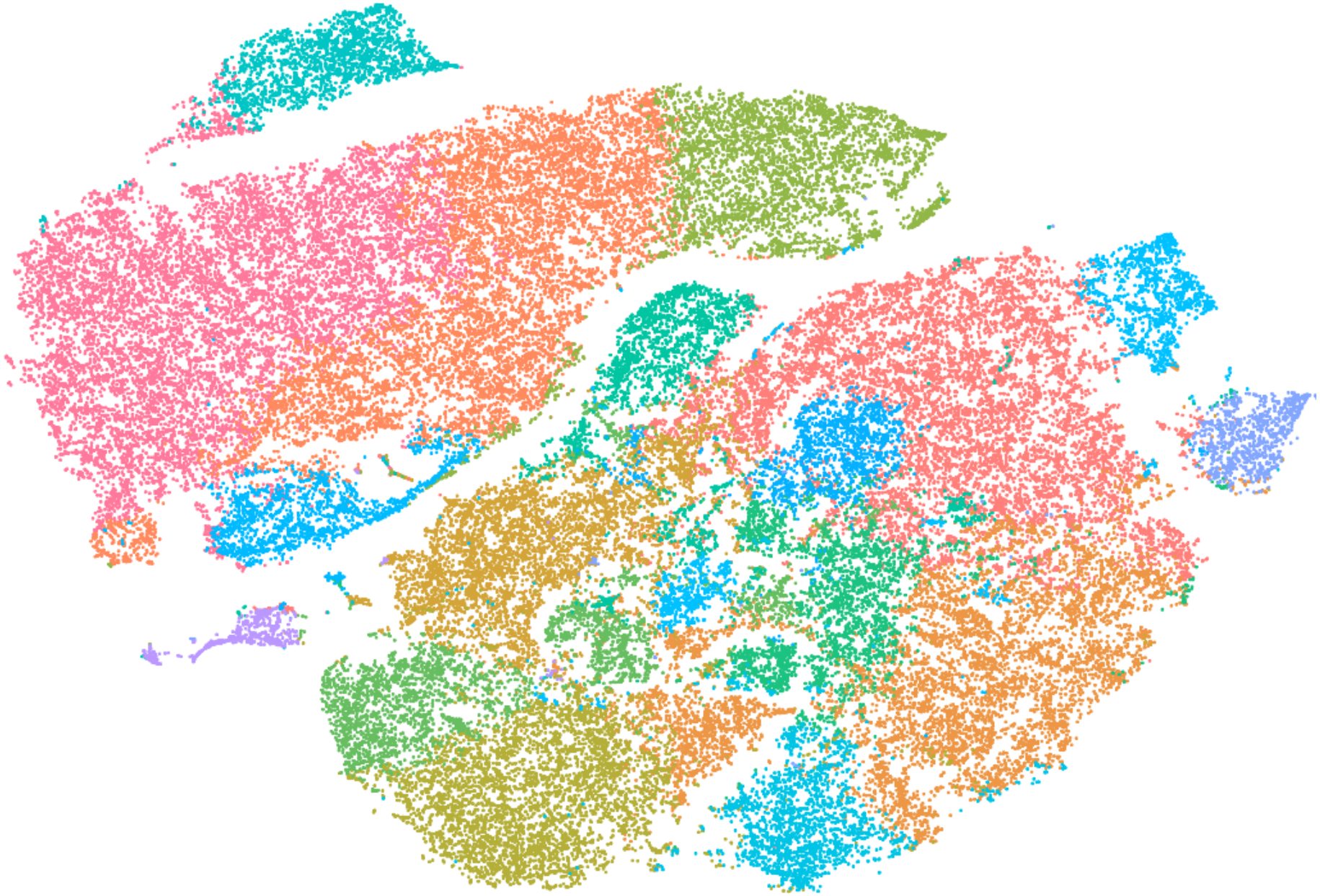
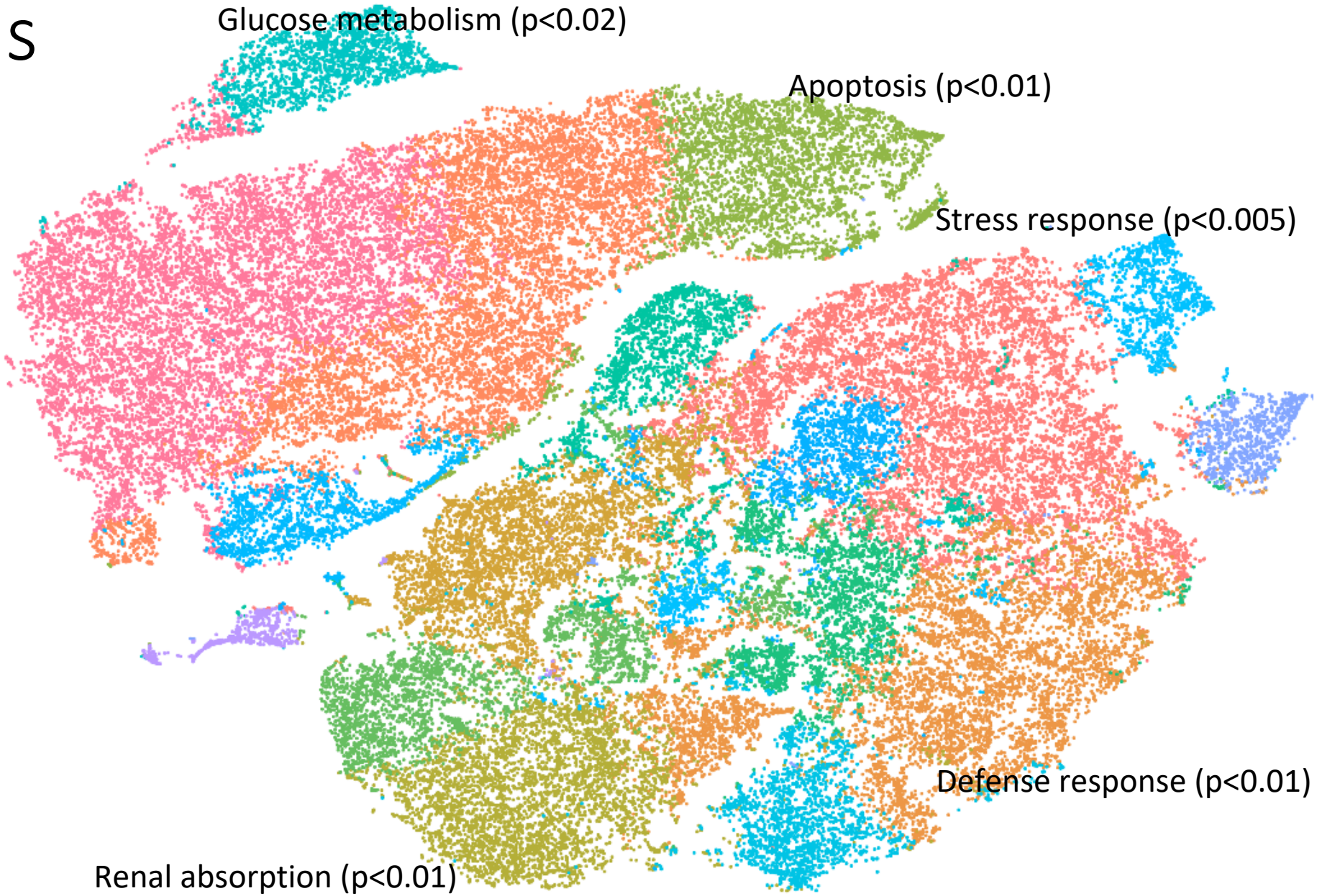
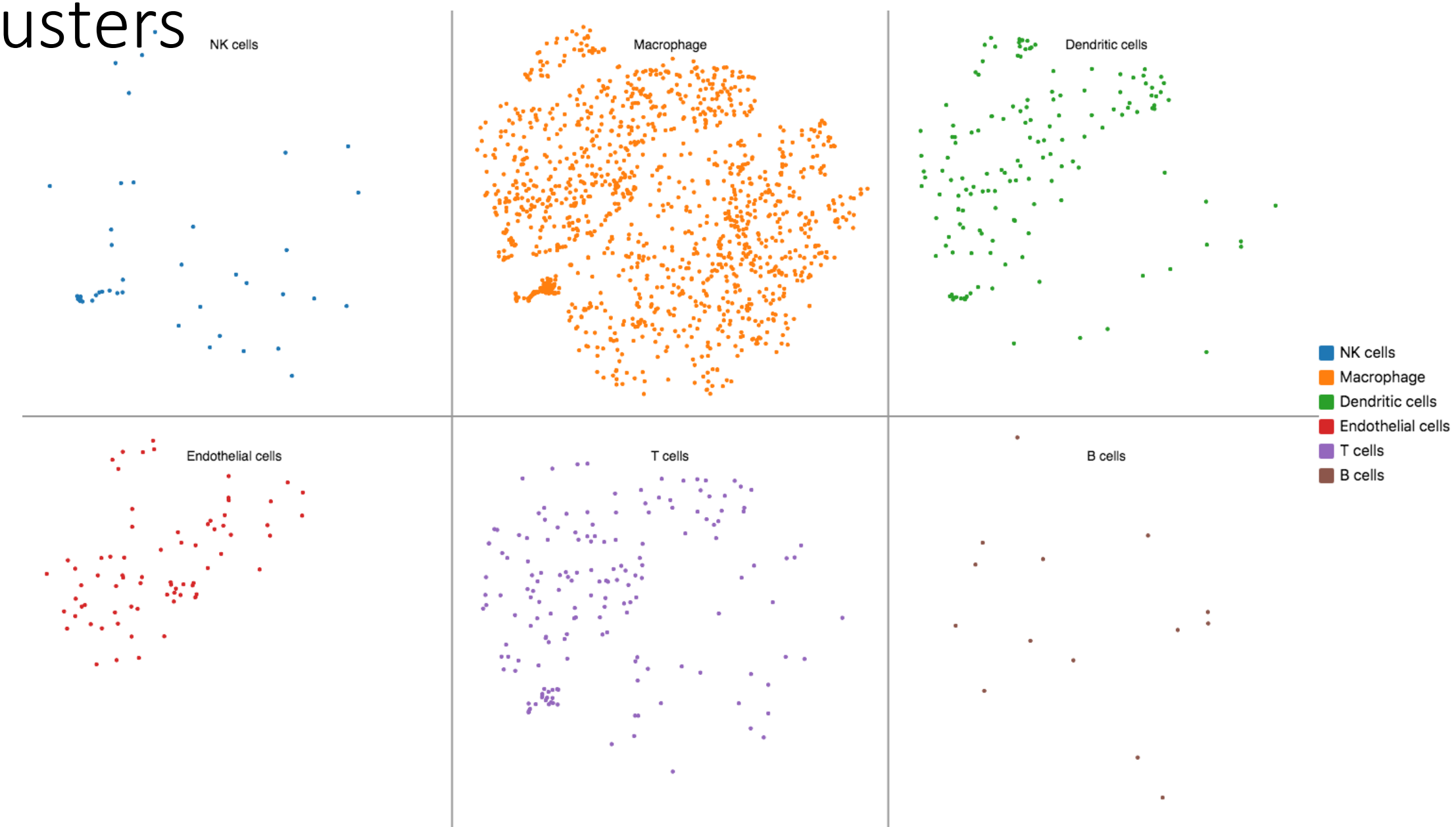
The landscape of low grade bladder cancer (~65000 cells)



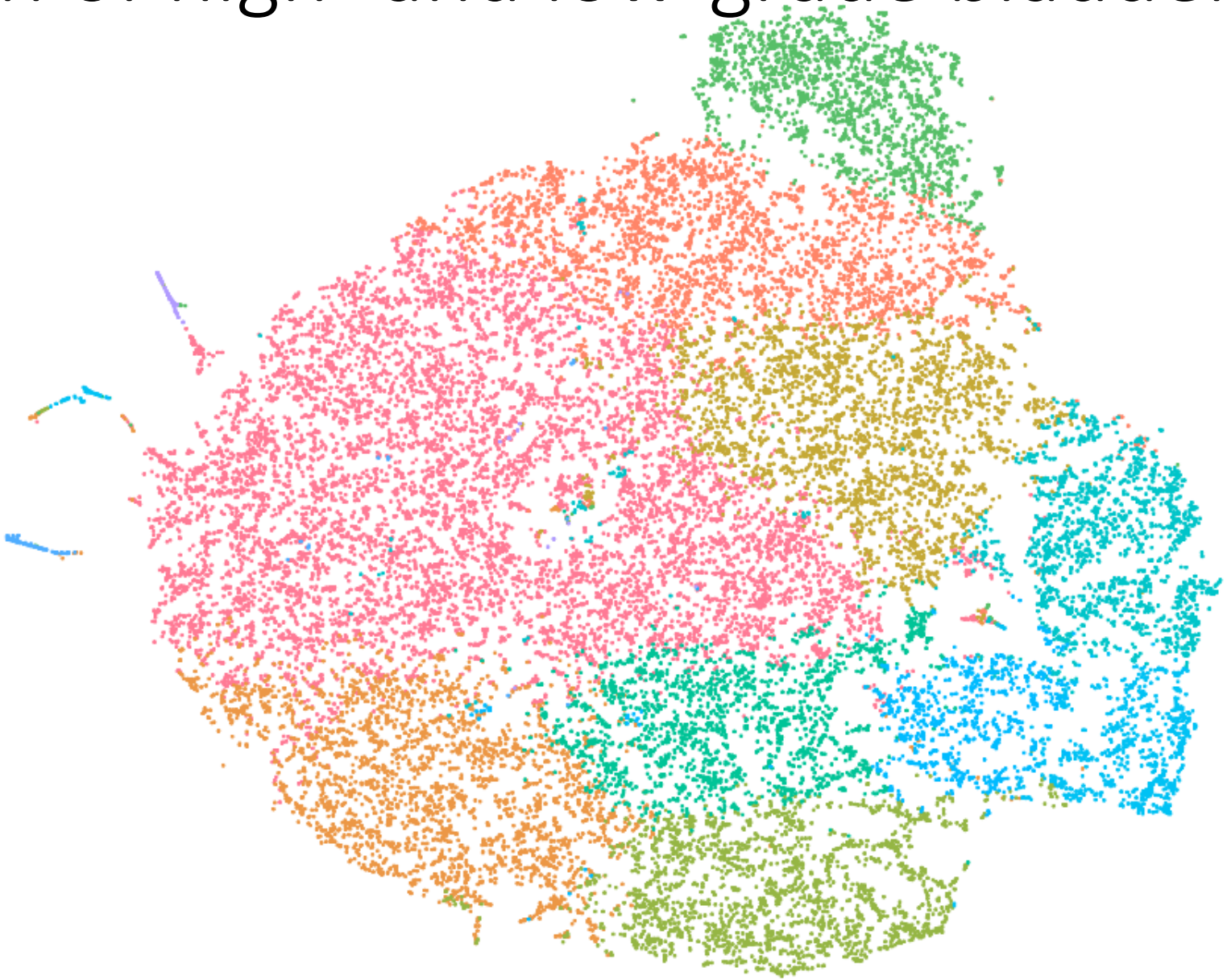
Gene expression pathway analysis of functional subpopulations



Diffuse immune cell distributions throughout the clusters



Comparison of high- and low-grade bladder specimens



Gene expression pathway differences between high and low grade tumor specimens

Pathway (GO)	Fold Enrichment	p-value
Oxygen transport	335.840	0.004
Hydrogen peroxide catabolic process	251.880	0.003
Positive regulation of cell death	173.710	0.005
Bicarbonate transport	114.491	0.008
Response to hydrogen peroxide	98.776	0.009
Protein heterooligomerization	75.188	0.012
Cellular oxidant detoxification	71.966	0.012

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Future Directions

- Deep mutational sequencing
- Dissection of tumor heterogeneity
- In depth metabolic phenotyping
- Correlation of tumor phenotype to chemotherapy or immune response.

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Conclusions

- Single cell analysis of NMIBC is feasible
- Immune infiltrates in these tumor samples are detectable and can be characterized
- Heterogenetic function within cell clusters can be detected
- Early evidence of metabolic distinctions between high grade and low grade disease

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